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Serrero

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(54) **COMPOSITIONS AND METHODS FOR RESTORING SENSITIVITY OF TUMOR CELLS TO ANTITUMOR THERAPY AND INDUCING APOPTOSIS**

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A61K 39/395 (2006.01)

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(58) **Field of Classification Search** 424/130.1, 424/138.1, 141.1, 142.1, 155.1, 156.1
See application file for complete search history.

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(57) **ABSTRACT**

Methods and compositions for restoring sensitivity to the antitumorigenic effects of antiestrogen therapy and/or cytotoxic therapy and inducing cell apoptosis are provided. Contacting tumor cells to GP88 antagonists (e.g., anti-GP88 antibodies, anti-GP88 antisense nucleic acids, GP88 siRNA, and small molecules) induces apoptosis and restores sensitivity to the antitumorigenic effects of antiestrogen therapy and cytotoxic therapy.

7 Claims, 12 Drawing Sheets

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Mouse GP88 cDNA

CGG	GGA	CCC	CGA	CGC	AGA	CAG	ACC	ATG	TGG	GTC	CTG	ATG	AGC	TGG	CTG	46
								M	W	V	L	M	S	W	L	8
GCC	TTC	GCG	GCA	GGG	CTG	GTA	GCC	GGA	ACA	CAG	TGT	CCA	GAT	GGG	CAG	94
A	F	A	A	G	L	V	A	G	T	Q	C	P	D	G	Q	24
TTC	TGC	CCT	GTT	GCC	TGC	TGC	CTT	GAC	CAG	GGA	GGA	GCC	AAC	TAC	AGC	142
F	C	P	V	A	C	C	L	D	Q	G	G	A	N	Y	S	40
TGC	TGT	AAC	CCT	CTT	CTG	GAC	ACA	TGG	CCT	AGA	ATA	ACG	AGC	CAT	CAT	190
C	C	N	P	L	L	D	T	W	P	R	I	T	S	H	H	56
CTA	GAT	GGC	TCC	TGC	CAG	ACC	CAT	GGC	CAC	TGT	CCT	GCT	GGC	TAT	TCT	238
L	D	G	S	C	Q	T	H	G	H	C	P	A	G	Y	S	72
TGT	CTT	CTC	ACT	GTG	TCT	GGG	ACT	TCC	AGC	TGC	TGC	CCG	TTC	TCT	AAG	286
C	L	L	T	V	S	G	T	S	S	C	C	P	F	S	K	88
GGT	GTG	TCT	TGT	GGT	GAT	GGC	TAC	CAC	TGC	TGC	CCC	CAG	GGC	TTC	CAC	334
G	V	S	C	G	D	G	Y	H	C	C	P	Q	G	F	H	104
TGT	AGT	GCA	GAT	GGG	AAA	TCC	TGC	TTC	CAG	ATG	TCA	GAT	AAC	CCC	TTG	382
C	S	A	D	G	K	S	C	F	Q	M	S	D	N	P	L	120
GGT	GCT	GTC	CAG	TGT	CCT	GGG	AGC	CAG	TTT	GAA	TGT	CCT	GAC	TCT	GCC	430
G	A	V	Q	C	P	G	S	Q	F	E	C	P	D	S	A	136
ACC	TGC	TGC	ATT	ATG	GTT	GAT	GGT	TCG	TGG	GGA	TGT	TGT	CCC	ATG	CCC	478
T	C	C	I	M	V	D	G	S	W	G	C	C	P	M	P	152
CAG	GCC	TCT	TGC	TGT	GAA	GAC	AGA	GTG	CAT	TGC	TGT	CCC	CAT	GGG	GCC	526
Q	A	S	C	C	E	D	R	V	H	C	C	P	H	G	A	168
TCC	TGT	GAC	CTG	GTT	CAC	ACA	CGA	TGC	GTT	TCA	CCC	ACG	GGC	ACC	CAC	574
S	C	D	L	V	H	T	R	C	V	S	P	T	G	T	H	184
ACC	CTA	CTA	AAG	AAG	TTC	CCT	GCA	CAA	AAG	ACC	AAC	AGG	GCA	GTG	TCT	622
T	L	L	K	K	F	P	A	Q	K	T	N	R	A	V	S	200
TTG	CCT	TTT	TCT	GTC	GTG	TGC	CCT	GAT	GCT	AAG	ACC	CAG	TGT	CCC	GAT	670
L	P	F	S	V	V	C	P	D	A	K	T	Q	C	P	D	216

FIG. 1A

Mousé GP88 cDNA (continued)

GAT TCT ACC TGC TGT GAG CTA CCC ACT GGG AAG TAT GGC TGC TGT CCA	718
D S T C C E L P T G K Y G C C P	232
ATG CCC AAT GCC ATC TGC TGT TCC GAC CAC CTG CAC TGC TGC CCC CAG	766
M P N A I C C S D H L H C C P Q	248
GAC ACT GTA TGT GAC CTG ATC CAG AGT AAG TGC CTA TCC AAG AAC TAC	814
D T V C D L I Q S K C L S K N Y	264
ACC ACG GAT CTC CTG ACC AAG CTG CCT GGA TAC CCA GTG AAG GAG GTG	862
T T D L L T K L P G Y P V K E V	280
AAG TGC GAC ATG GAG GTG AGC TGC CCT GAA GGA TAT ACC TGC TGC CGC	910
K C D M E V S C P E G Y T C C R	296
CTC AAC ACT GGG GCC TGG GGC TGC TGT CCA TTT GCC AAG GCC GTG TGT	958
L N T G A W G C C P F A K A V C	312
TGT GAG GAT CAC ATT CAT TGC TGC CCG GCA GGG TTT CAG TGT CAC ACA	1006
C E D H I H C C P A G F Q C H T	328
GAG AAA GGA ACC TGC GAA ATG GGT ATC CTC CAA GTA CCC TGG ATG AAG	1054
E K G T C E X G I L Q V P W M <u>K</u>	344
AAG GTC ATA GCC CCC CTC CGC CTG CCA GAC CCA CAG ATC TTG AAG AGT	1102
<u>K V I A P L R L P D P Q I L K S</u>	360
GAT ACA CCT TGT GAT GAC TTC ACT AGG TGT CCT ACA AAC AAT ACC TGC	1150
<u>D T</u> P C D D F T R C P T N N T C	376
TGC AAA CTC AAT TCT GGG GAC TGG GGC TGC TGT CCC ATC CCA GAG GCT	1198
C K L N S G D W G C C P I P E A	392
GTC TGC TGC TCA GAC AAC CAG CAT TGC TGC CCT CAG GGC TTC ACA TGT	1246
V C C S D N Q H C C P Q G F T C	408
CTG GCT CAG GGG TAC TGT CAG AAG GGA GAC ACA ATG GTG GCT GGC CTG	1294
L A Q G Y C Q K G D T M V A G L	424
GAG AAG ATA CCT GCC CGC CAG ACA ACC CCG CTC CAA ATT GGA GAT ATC	1342
E K I P A R Q T T P L Q I G D I	440

FIG. 1B

Mouse GP88 cDNA (continued)

GGT TGT GAC CAG CAT ACC AGC TGC CCA GTA GGG CAA ACC TGC TGC CCA	1390
G C D Q H T S C P V G Q T C C P	456
AGC CTC AAG GGA AGT TGG GCC TGC TGC CAG CTG CCC CAT GCT GTG TGC	1438
S L K G S W A C C Q L P H A V C	472
TGT GAG GAC CGG CAG CAC TGT TGC CCG GCC GGG TAC ACC TGC AAC GTG	1486
C E D R Q H C C P A G Y T C N V	488
AAG GCG AGG ACC TGT GAG AAG GAT GTC GAT TTT ATC CAG CCT CCC GTG	1534
K A R T C E K D V D F I Q P P V	504
CTC CTG ACC CTC GGC CCT AAG GTT GGG AAT GTG GAG TGT GGA GAA GGG	1582
L L T L G P K V G N V E C G E G	520
CAT TTC TGC CAT GAT AAC CAG ACC TGT TGT AAA GAC AGT GCA GGA GTC	1630
H F C H D N Q T C C K D S A G V	536
TGG GCC TGC TGT CCC TAC CTA AAG GGT GTC TGC TGT AGA GAT GGA CGT	1678
W A C C P Y L K G V C C R D G R	552
CAC TGT TGC CCC GGT GGC TTC CAC TGT TCA GCC AGG GGA ACC AAG TGT	1726
H C C P G G F H C <u>S A R G T K C</u>	568
TTG CGA AAG AAG ATT CCT CGC TGG GAC ATG TTT TTG AGG GAT CCG GTC	1774
<u>L R K K I P R</u> W D M F L R D P V	584
CCA ACA CCG CTA CTG TAA GGA AGG GCT ACA GAC TTA AGG AAC TCC ACA	1822
P R P L L *	589
GTC CTG GGA ACC CTG TTC CGA GGG TAC CCA CTA CTC AGG CCT CCC TAG	1870
CGC CTC CTC CCC TAA CGT CTC CCC GGC CTA CTC ATC CTG AGT CAC CCT	1918
ATC ACC ATG GGA GGT GGA GCC TCA AAC TAA AAC CTT CTT TTA TGG AAA	1966
GAA GGC TGT GGC CAA AAG CCC CGT ATC AAA CTG CCA TTT CTT CCG GTT	2014
TCT GTG GAC CTT GTG GCC AGG TGC TCT TCC CGA GCC ACA GGT GTT CTG	2062
TGA GCT TGC TTG TGT GTG TGT GCG CGT GTG CGT GTG TTG CTC <u>QAA TAA</u>	2110
<u>AGT</u> TTG TAC GCT TTC TGA AAA AAA AAA	2137

FIG. 1C

Nucleotide sequence of human granulin/epithelin precursor (human GP88).
Human Granulin Genbank M75161\$

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[gcaggcaga ccatgtggac cttggtgagc tgggtggcct taacagcagg gctggtggct
ggaacgcggt gccagatgg tcagttctgc cctgtggcct gctgcctgga ccccggagga
gccagctaca gctgctgccg tccccttctg gacaaatggc ccacaacact gagcaggcat
ctgggtggcc cctgccaggt tgatgccac tgctctgccg gccactcctg catctttacc
gtctcagga cttccagttg ctgcccctc ccagaggccg tggcatgccg ggatggccat
cactgctgcc cacggggctt ccactgcagt gcagacggga gatcctgctt ccaaagatca
ggtaacaact ccgtgggtgc catccagtgc cctgatagtc agttcgaatg cccggacttc
tccacgtgct gtgttatggt cgatggctcc tgggggtgct gcccctatgcc ccaggcttcc
tgctgtgaag acagggtgca ctgctgtccg cacgggtgcct tctgcgacct gggtcacacc
cgctgcatca caccacggg caccacccc ctggcaaaga agctccctgc ccagaggact
aacagggcag tggccttgtc cagctcggtc atgtgtccgg acgcacggtc ccggtgcctt
gatggttcta cctgctgtga gctgccctg ggaagtatg gctgctgcc aatgcccac
gccacctgct gctccgatca cctgcactgc tgcccccaag acactgtgtg tgacctgatc
cagagtaagt gcctctcaa ggagaacgct accacggacc tctcactaa gctgcctgcg
cacacagtgg gcgatgtgaa atgtgacatg gaggtgagct gccagatgg ctatacctgc
tgccgtctac agtcgggggc ctggggctgc tgccccttta cccaggctgt gtgctgtgag
gaccacatac actgctgtcc cgcgggggtt acgtgtgaca cgcagaaggg tacctgtgaa
caggggcccc accaggtgcc ctggatggag aaggccccag ctcacctcag cctgccagac
ccacaagcct tgaagagaga tgtcccctgt gataatgtca gcagctgtcc ctctccgat
acctgctgcc aactcacgtc tggggagtgg ggetgctgtc caatcccaga ggctgtctgc
tgctcggacc accagcactg ctgccccag cgatacacgt gtgtagctga ggggcagtgt
cagcgaggaa gcgagatcgt ggctggactg gagaagatgc ctgcccgccg cggttcctta
tcccaccca gagacatcgg ctgtgaccag cacaccagct gcccggtggg cggaacctgc
tgcccagacc aggggtgggag ctgggcctgc tgccagttgc cccatgctgt gtgctgcgag
gatcggcagc actgctgcc ggctggctac acctgcaacg tgaaggctcg atcctgcgag
aaggaagtgg tctctgccc gcctgccacc ttctggccc gtagecctca cgtgggtgtg
aaggacgtgg agtgtgggga aggacacttc tgccatgata accagacctg ctgccgagac
aaccgacagg gctgggcctg ctgtccctac gccagggcg tctgttgtgc tgatcggcgc
cactgctgtc ctgctggctt ccgctgcgca cgcaggggta ccaagtgtt gcgcagggag
gccccgcgct gggacgcccc tttagaggac ccagccttga gacagctgct gtgagggaca
gtactgaaga ctctgcagcc ctgggacc cactcggagg gtgccctctg ctcaggcctc
gtactgaaga ctctgcagcc ctgggacc cactcggagg gtgccctctg ctcaggcctc
cctagcacct ccccctaacc aaattctccc tggaccccat tctgagctcc ccatccat
gggaggtggg gcctcaatct aaggccctc cctgtcagaa gggggtgag gcaaaagccc
attacaagct gccatcccct ccccgttca gtggacctg tggccaggtg cttttcccta
tccacagggg tgtttgtgtg ttgggtgtgc tttcaataaa gtttgcact ttctt*

```

FIG. 2A

Amino-acid sequence of human granulin/epithelin precursor (human GP88).

MWTLVSWVALTAGLVAGTRCPDGQFCPVACCLDPGGASYSCCRP
LLDKWPTTLSRHLGGPCQVDAHCSAGHSCIFTVSGTSSCCPFPEAVACGDGHHCCPRG
FHCSADGRSCFQRSGNNSVGAIQCPDSQFECPDFSTCCVMVDGSWGCCPMPQASCCED
RVHCCPHGAFCDLVHTRCITPTGTHPLAKKLPAQRTNRAVALSSVMCPDARSRCPDG
STCCELPSGKYGCCPMPNATCCSDHLHCCPQDTVCDLIQSKLSKENATTDLLTYLPA
HTVGDVKCDMEVSCP DGYTCCRLQSGAWGCCPFTQAVCCEDHIHCCPAGFTCDTQKGT
CEQGPHQVPWMEKAPAHLSLPDPOALKRDVPCDNVSSCPSSDTCCQLTSGEWGCCPIP
EAVCCSDHQHCCPQRYTCVAEGQCQRGSEIVAGLEKMPARRGSLSHPRDIGCDQHTSC
PVGGTCCPSQGGSWACCQLPHAVCCEDRQHCCPAGYTCNVKARSCEKEVVSAQPATFL
ARSPHVGVKDVECGEGHFCHDNQTCCRDNRQGWACCPYAQGVCCADRRHCCPAGFRCA
RRGTKCLRREAPRWDAPLRDPALRQLL*

FIG. 2B

GP88 cDNA CLONE in SK



STRUCTURE OF pCMV₄ EXPRESSION VECTOR

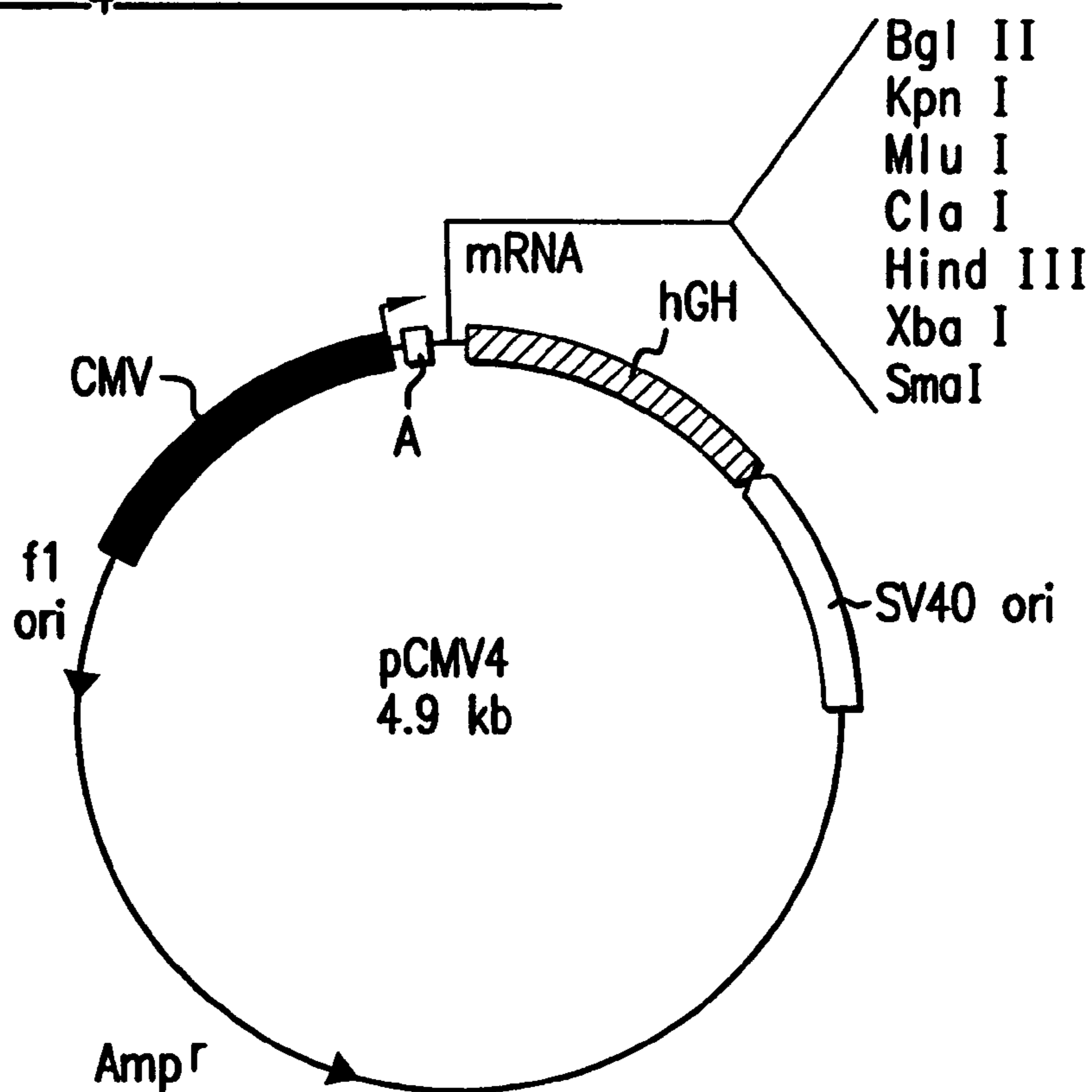


FIG. 4

GP88 inhibits apoptosis in MCF-7 cells in response to tamoxifen

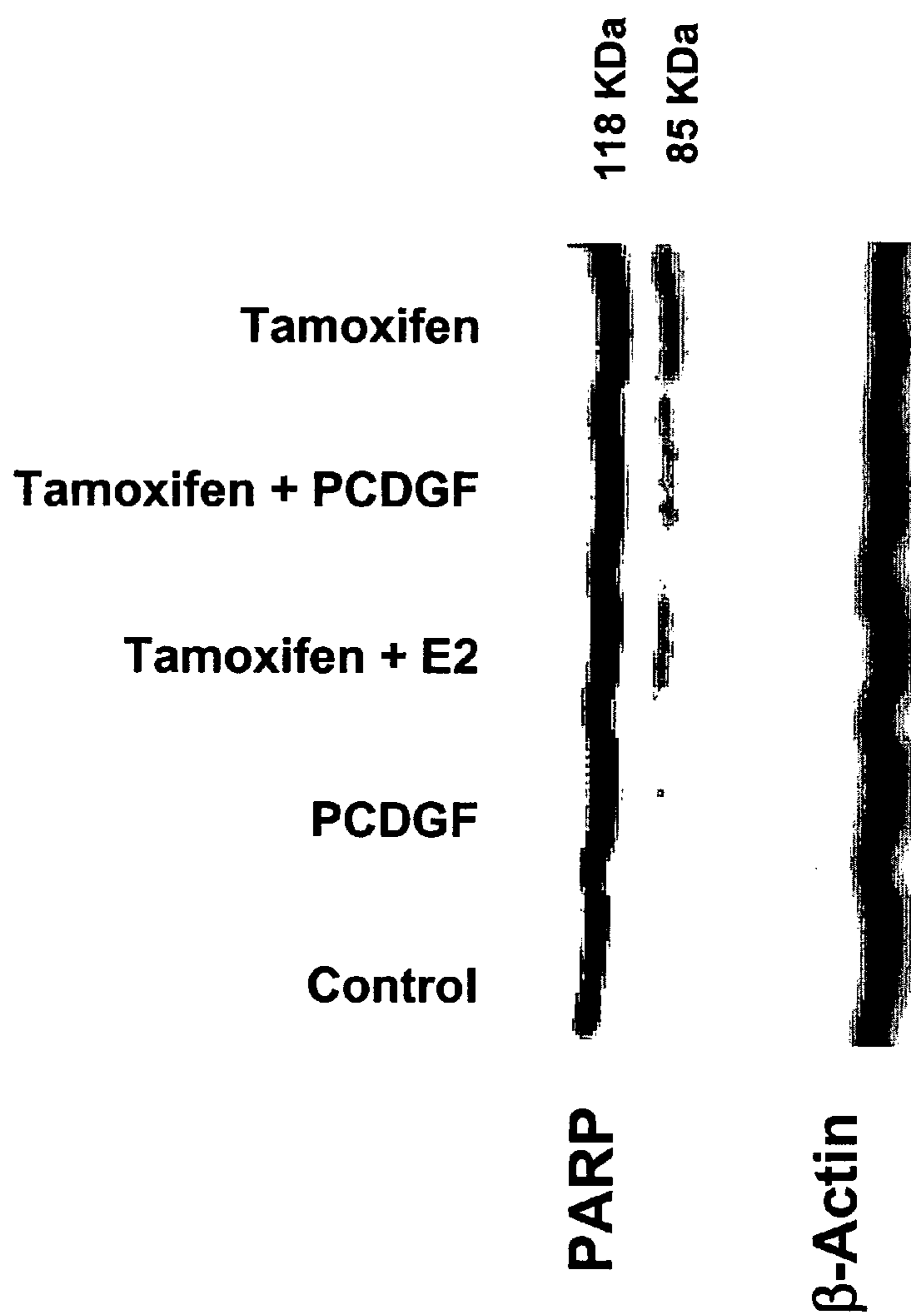
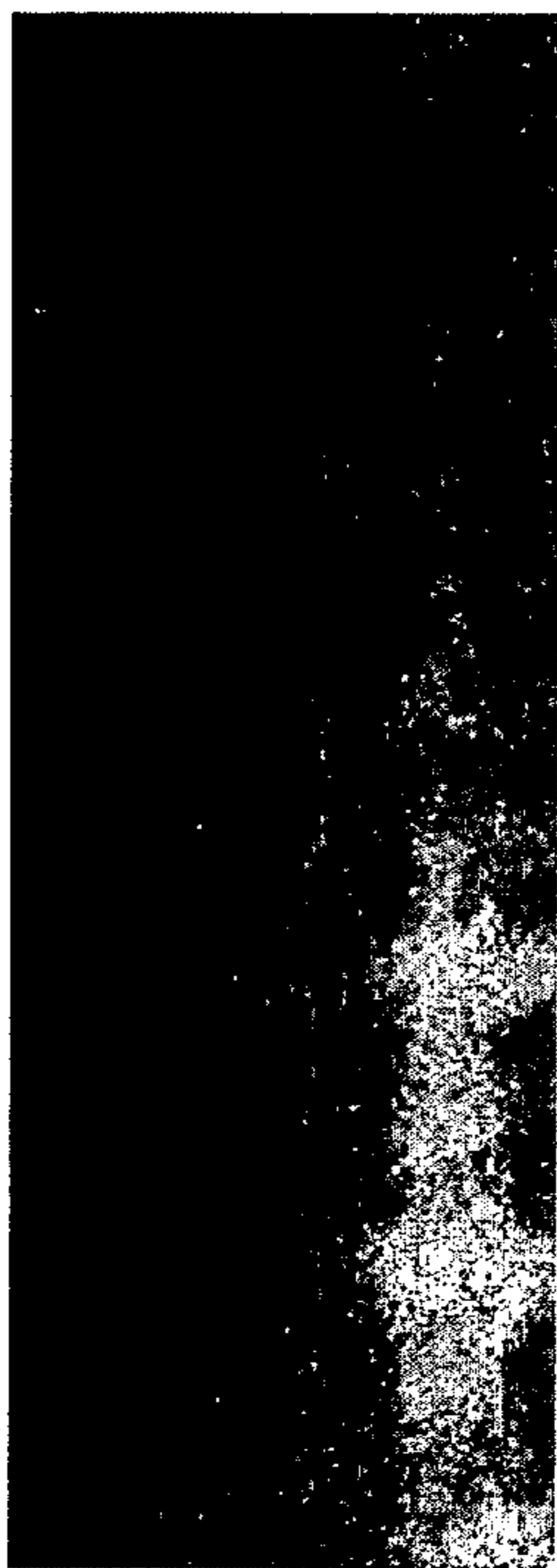
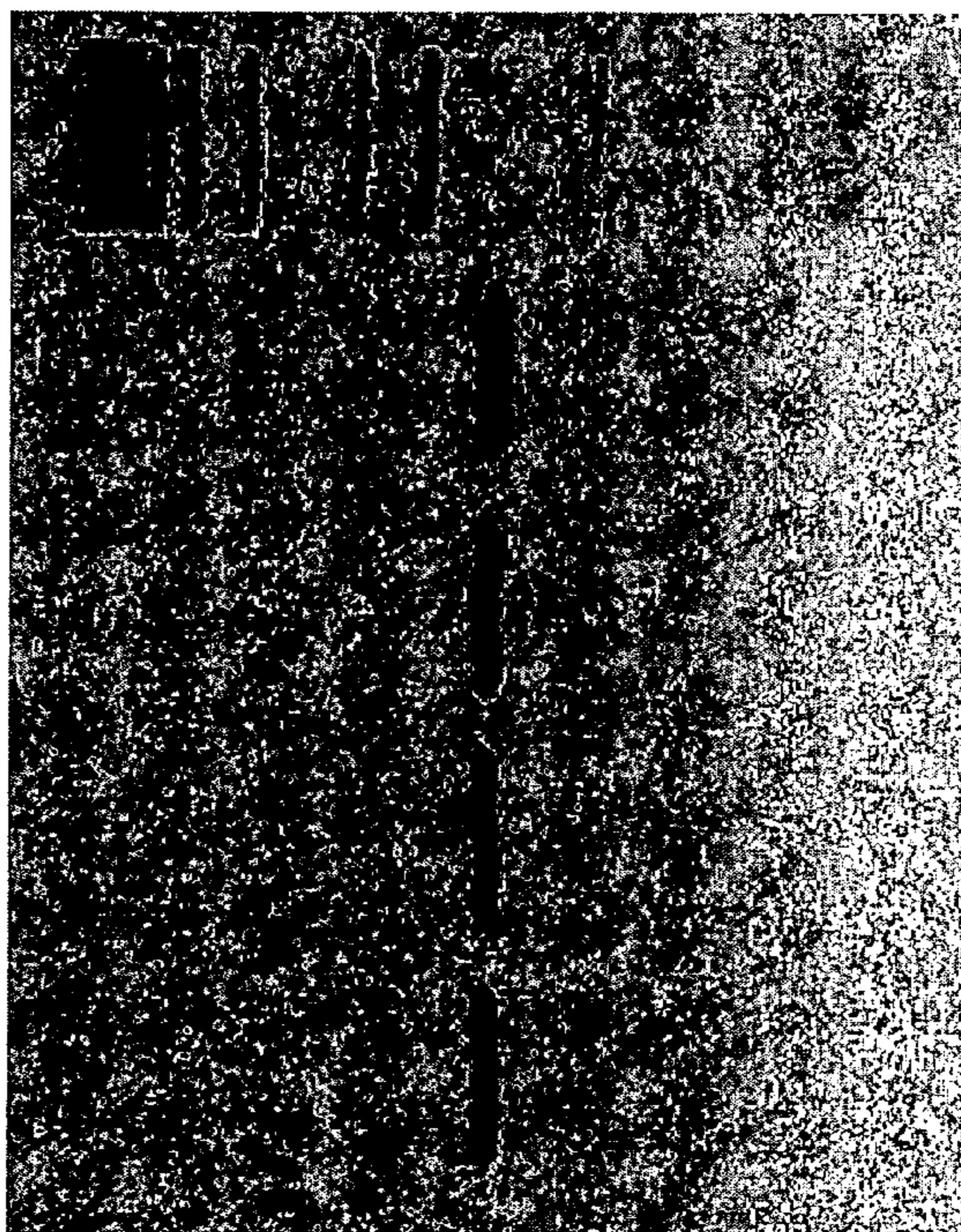


FIG. 5

5B4+T T 5B4 C



Bcl-2



GAPD

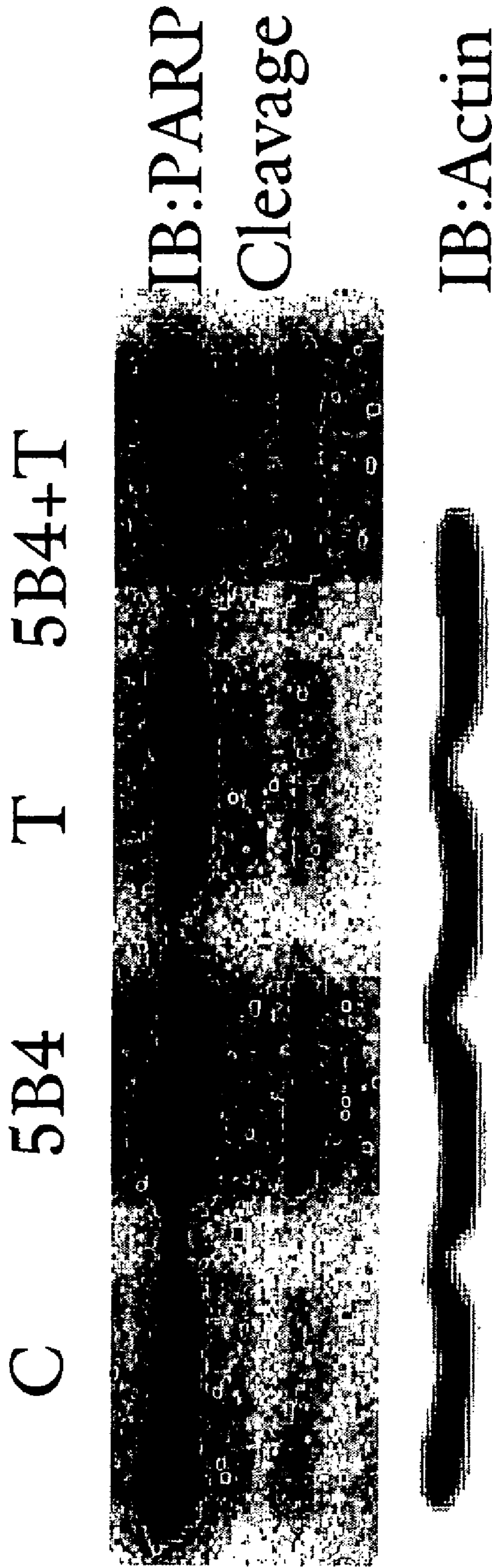
Tamoxifen resistant MCF-7 cells

FIG. 6



O4 cells (GP88 overexpressing tamoxifen resistant MCF-7 cells)

FIG. 7



Tamoxifen resistant MCF-7 cells

FIG. 8

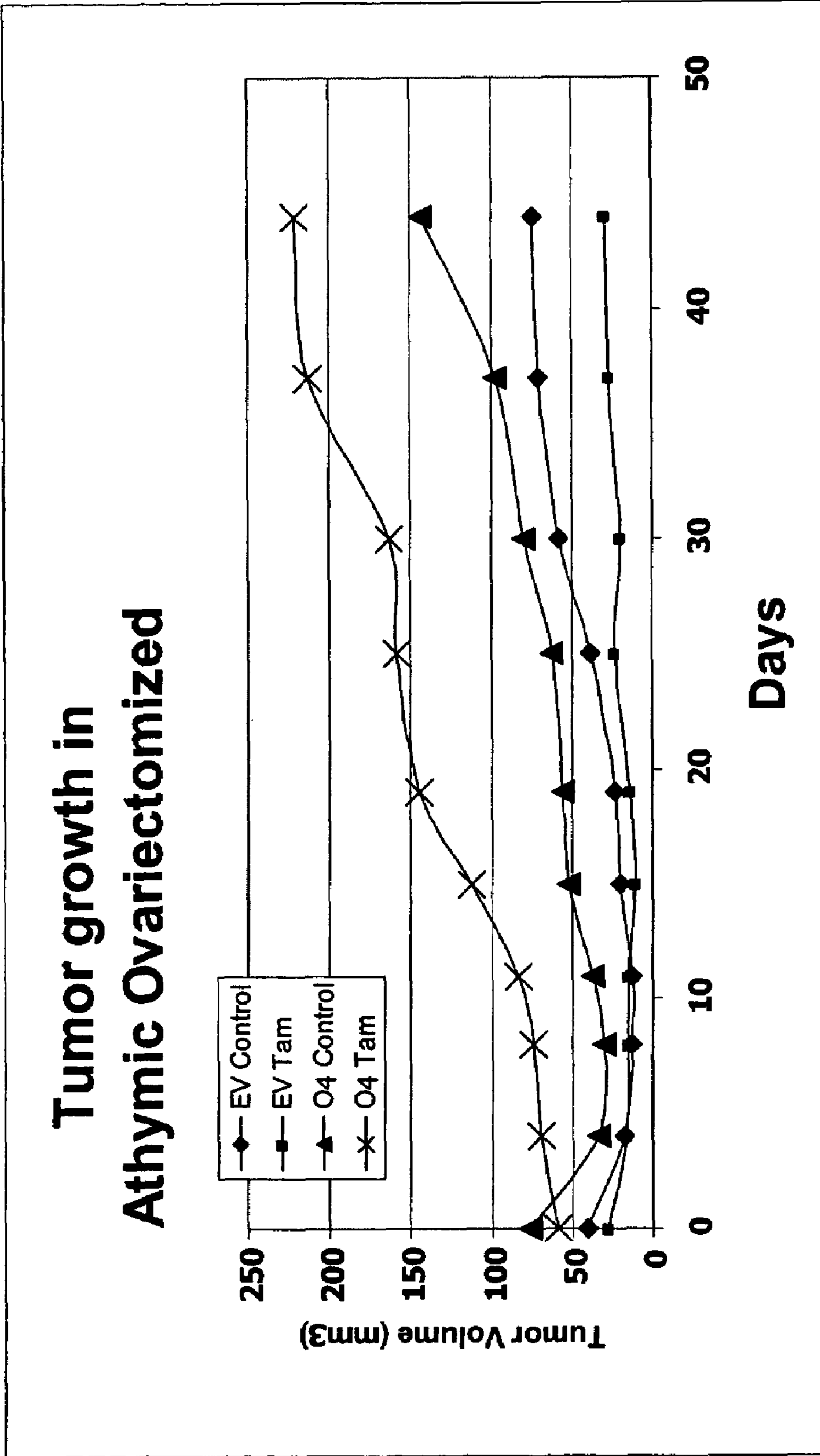


FIG. 9

**COMPOSITIONS AND METHODS FOR
RESTORING SENSITIVITY OF TUMOR
CELLS TO ANTITUMOR THERAPY AND
INDUCING APOPTOSIS**

CROSS-REFERENCE TO RELATED
APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/480/439, filed Jun. 23, 2003. The following U.S. Patents and U.S. Patent Publications are expressly incorporated by reference herein in their entirety: U.S. Pat. No. 6,720,159; U.S. Pat. No. 6,309,826; U.S. patent Publication No. 2003/0099646; U.S. patent Publication No. 2003/0215445; and U.S. patent Publication No. 2002/0025543.

FIELD OF THE INVENTION

This invention relates to cell biology, physiology and medicine, and concerns an 88 kDa glycoprotein growth factor ("GP88") and compositions and methods which affect the expression and biological activity of GP88. This invention also relates to kit products, compositions and methods which are useful for diagnosis and treatment of diseases including cancer.

BACKGROUND OF THE INVENTION

The proliferation and differentiation of cells in multicellular organisms is subject to a highly regulated process. A distinguishing feature of cancer cells is the absence of control over this process; proliferation and differentiation become deregulated resulting in uncontrolled growth. Significant research efforts have been directed toward better understanding this difference between normal and tumor cells. One area of research focus is growth factors and, more specifically, autocrine growth stimulation.

Growth factors are polypeptides which carry messages to cells concerning growth, differentiation, migration and gene expression. Typically, growth factors are produced in one cell and act on another cell to stimulate proliferation. However, certain malignant cells, in culture, demonstrate a greater or absolute reliance on an autocrine growth mechanism. Malignant cells which observe this autocrine behavior circumvent the regulation of growth factor production by other cells and are therefore unregulated in their growth.

Study of autocrine growth control advances understanding of cell growth mechanisms and can lead to important advances in the diagnosis and treatment of cancer. Toward this end, a number of growth factors have been studied, including insulin-like growth factors ("IGF1" and "IGF2"), gastrin-releasing peptide ("GRP"), transforming growth factors alpha and beta ("TGF-a" and "TGF-b"), and epidermal growth factor ("EGF").

The present invention is directed to a recently discovered growth factor. This growth factor was first discovered in the culture medium of highly tumorigenic "PC cells," an insulin-independent variant isolated from the teratoma derived adipogenic cell line 1246. This growth factor is referred to herein as "GP88" or "GP88." GP88 has been purified and structurally characterized. Amino acid sequencing of GP88 indicates that GP88 has amino acid sequence similarities with the mouse granulin/epithelin precursor.

Granulins/epithelins ("grn/epi") are 6kDa polypeptides and belong to a novel family of double cysteine rich polypeptides. U.S. Pat. No. 5,416,192 (Shoyab et al.) is

directed to 6 kDa epithelins, particularly epithelin 1 and epithelin 2. According to Shoyab, both epithelins are encoded by a common 63.5 kDa precursor, which is processed into smaller forms as soon as it is synthesized, so that the only natural products found in biological samples are the 6 kDa forms. Shoyab et al. teaches that the epithelin precursor is biologically inactive.

Contrary to the teachings of Shoyab et al., the inventor's laboratory has demonstrated that the precursor is not always processed as soon as it is synthesized. Studies, conducted in part by this inventor, have demonstrated that the precursor (i.e., GP88) is in fact secreted as an 88kDa glycoprotein with an N-linked carbohydrate moiety of 20 kDa. Analysis of the N-terminal sequence of GP88 indicates that GP88 starts at amino acid 17 of the grn/epi precursor, demonstrating that the first 17 amino acids from the protein sequence deduced from the precursor cDNA correspond to a signal peptide compatible with targeting for membrane localization or for secretion. Also in contrast to the teachings of Shoyab et al., GP88 is biologically active and has growth promoting activity, particularly as an autocrine growth factor for the producer cells.

Multi-cellular organisms require a careful balance between the production and destruction of cells in tissues throughout the body. Apoptosis, or programmed cell death, is a controlled process by which damaged cells or cells replicating outside of normal cellular control can be eliminated without causing the tissue destruction and inflammatory responses often associated with acute injury and necrosis.

Recent studies indicate that apoptosis is controlled through a metabolic pathway which may be induced by a variety of signals (e.g., hormones, serum growth factor deprivation, chemotherapeutic agents, ionizing radiation, and viral infection). See, e.g., U.S. Pat. Nos. 6,586,395 and 6,570,002. The Bcl-2 family of genes regulate apoptosis in many cell types. The normal function of Bcl-2 is to block apoptosis in response to a variety of signals (e.g., radiation, hyperthermia, growth factor withdrawal, glucocorticoids, and multiple classes of chemotherapeutic agents). Id. Thus, blocking the activity of Bcl-2 induces apoptosis. Zhang et al., *Clinical Cancer Research*, 5:2971-2977 (October 1999). For example, the anti-estrogen compound tamoxifen down regulates Bcl-2 and thus induces apoptosis in breast cancer cells. Id. In addition to inhibiting the growth promoting effect of estrogen, tamoxifen has also been shown to induce programmed cell death in breast cancer cell lines and in clinical samples. Failure to undergo apoptosis in response to tamoxifen confers tamoxifen resistance.

Anti-estrogen therapy is widely used for the treatment of breast cancer. Tamoxifen has been the major agent used for this purpose. The activity of tamoxifen is typically observed in breast tumors that are estrogen receptor positive, since estrogen is the major growth stimulator for these types of tumors. However, after prolonged anti-hormonal therapy, breast cancer can progress from an estrogen sensitive to insensitive state. Breast tumors that were previously growth inhibited by tamoxifen and other anti-estrogen compounds then become resistant to anti-estrogen treatment.

The morphological changes induced by tamoxifen are characteristic of the changes induced by apoptosis. Up-regulation of Bcl-2 by HER2 suppresses tamoxifen-induced apoptosis in breast cancer cells. Kumar et al., *Clin. Cancer Res.* 1996 Jul.2(7):1215-9. Thus, modulation of Bcl-2 levels provides a mechanism for inducing apoptosis. Inducing apoptosis leads to tumor regression by eliminating, shrinking, and destroying tumor cells. Trauth et al., *Science*. 1989

Jul. 21;245(4915):301-5. Administration of antisense oligonucleotides directed to the anti-apoptotic Bcl-2 gene induces tumor regression in mice in vivo. Elez et al., *Oncogene* (2003) 22: 69-80.

What is needed are new methods and compositions for inducing apoptosis and restoring sensitivity to the antitumorigenic effects of antiestrogen therapy and cytotoxic therapy.

BRIEF SUMMARY OF THE INVENTION

The inventor has unexpectedly discovered that a glycoprotein (GP88), which is expressed in a tightly regulated fashion in normal cells, is overexpressed and unregulated in highly tumorigenic cells derived from the normal cells, that GP88 acts as a stringently required growth stimulator for the tumorigenic cells and that inhibition of GP88 expression or action in the tumorigenic cells results in an inhibition of the tumorigenic properties of the overproducing cells.

In one embodiment of the invention, sensitivity to the antitumorigenic effects of antiestrogens and/or cytotoxic compounds is restored by administering a GP88 antagonist to a tumor cell. In another embodiment, the GP88 antagonist can be administered together with or sequentially with an antiestrogen or cytotoxic (e.g., chemotherapeutic) compound. Tumor cells overexpressing GP88 are growth stimulated and are resistant to treatment by tamoxifen and other anti-estrogens. Administration of a GP88 antagonist to a tumor cell restores sensitivity to (1) tamoxifen and other antiestrogens (e.g., raloxifene, aromatase inhibitors) and/or (2) cytotoxic or chemotherapeutic agents (e.g., Altretamine, Bleomycin, Busulphan, Calcium Folate, Capecitabine, Carboplatin, Carmustine, Chlorambucil, Cisplatin, Cladribine, Crisantaspase, Cyclophosphamide, Cytarabine, Dacarbazine, Dactinomycin, Daunorubicin, Docetaxel, Doxorubicin, Epirubicin, Etoposide, Fludarabine, Fluorouracil, Gemcitabine, Hydroxyurea, Idarubicin, Ifosfamide, Irinotecan, Liposomal doxorubicin, Lomustine, Melphalan, Mercaptopurine, Methotrexate, Mitomycin, Mitoxantrone, Oxaliplatin, Paclitaxel, Pentostatin, Procarbazine, Raltitrexed, Streptozocin, Tegafur-uracil, Temozolomide, Thiotepa, Tioguanine/Thioguanine, Topotecan, Treosulfan, Vinblastine, Vincristine, Vindesine, and Vinorelbine) and combinations thereof. GP88 antagonists can also be administered either before or after administration of an antiestrogen and/or a cytotoxic compound to restore sensitivity to the antiestrogen and/or cytotoxic compound.

The invention also provides methods and compositions for inducing apoptosis by administering a GP88 antagonist to a tumor cell. Induction of apoptosis can be determined, for example, by measuring the level of Bcl-2 protein or mRNA, measuring the ability of the cell to cleave PARP (poly (ADP ribose) polymerase), or evaluating the appearance or volume of the tumor cell or cells.

This invention also provides GP88 antagonizing compositions capable of inhibiting the expression or activity of GP88, methods for treating diseases associated with a defect in GP88 quantity or activity such as cancer, including, but not limited to, cancer in mammalian blood, cerebrospinal fluid, serum, plasma, prostate, bladder, nasopharynx, head and neck, cervix, neural tissue, thyroid, pancreas, urine, nipple aspirate, liver, kidney, breast, bone, bone marrow, testes, brain, neural, ovary, skin, and lung, methods for determining the susceptibility of a subject to diseases associated with a defect in GP88 expression or action, methods for measuring susceptibility to GP88 antagonizing therapy,

and methods, reagents, and kits for the in vitro and in vivo detection of GP88 and tumorigenic activity in cells.

Additional objects and advantages of the invention will be set forth in part in the description that follows, and in part will be obvious from the description, or may be learned by the practice of the invention.

The present invention also provides compositions and methods for diagnosis and treatment of diseases, such as breast cancer, in which cells exhibit an altered expression of GP88 or altered response to GP88. Use of the term "altered expression" herein means increased expression or overexpression of GP88 by a factor of at least two-fold, and at times by a factor of 10 or more, based on the level of mRNA or protein as compared to corresponding normal cells or surrounding peripheral cells. The term "altered expression" also means expression which became unregulated or constitutive without being necessarily elevated. Use of the terms increased or altered "response" to GP88 means a condition wherein increase in any of the biological functions (e.g., growth, differentiation, viral infectivity) conferred by GP88 results in the same or equivalent condition as altered expression of GP88.

Use of the term "GP88" or "PCDGF" herein means epithelin/granulin precursor (also known as progranulin), in cell extracts and extracellular fluids, and is intended to include not only GP88 according to the amino acid sequences included in FIGS. 1-3, which are of mouse and human origins, but also GP88 of other species. In addition, the term also includes functional derivatives thereof having additional components such as a carbohydrate moiety including a glycoprotein or other modified structures.

Also intended by the term GP88 or PCDGF is any polypeptide fragment having at least 10 amino-acids present in the above mentioned sequences. Sequences of this length are useful as antigens and for making immunogenic conjugates with carriers for the production of antibodies specific for various epitopes of the entire protein. Such polypeptides are useful in screening such antibodies and in the methods directed to detection of GP88 in biological fluids. It is well known in the art that peptides are useful in generation of antibodies to larger proteins. In one embodiment of this invention, it is shown that peptides from 12-19 amino-acids in length have been successfully used to develop antibodies that recognize the full length GP88.

The polypeptide of this invention may exist covalently or non-covalently bound to another molecule. For example, it may be fused to one or more other polypeptides via one or more peptide bonds such as glutathione transferase, polyhistidine, or myc tag.

The polypeptide is sufficiently large to comprise an antigenically distinct determinant or epitope which can be used as an immunogen to reproduce or test antibodies against GP88 or a functional derivative thereof.

One embodiment includes the polypeptide substantially free of other mammalian peptides. GP88 of the present invention can be biochemically or immunochemically purified from cells, tissues or a biological fluid. Alternatively, the polypeptide can be produced by recombinant means in a prokaryotic or eukaryotic expression system and host cells.

"Substantially free of other mammalian polypeptides" reflects the fact that the polypeptide can be synthesized in a prokaryotic or a non-mammalian or mammalian eukaryotic organism, if desired. Alternatively, methods are well known for the synthesis of polypeptides of desired sequences by chemical synthesis on solid phase supports and their subsequent separation from the support. Alternatively, the protein can be purified from tissues or fluids of mammals where it

naturally occurs so that it is at least 90% pure (on a weight basis) or even 99% pure, if desired, of other mammalian polypeptides, and is therefore substantially free from them. This can be achieved by subjecting the tissue extracts or fluids to standard protein purification such as on immuno-
 5 absorbants bearing antibodies reactive against the protein. One embodiment of the present invention describes purification methods for the purification of naturally occurring GP88 and of recombinant GP88 expressed in baculovirus infected insect cells, and in mammalian cells. Alternatively,
 10 purification from such tissues or fluids can be achieved by a combination of methods known in the art.

As an alternative to a native purified or recombinant glycoprotein or polypeptide, "GP88" is intended to also include functional derivatives. By functional derivative is meant a "fragment," "variant," "analog," or "chemical derivative" of the protein or glycoprotein as defined below. A functional derivative retains at least a portion of the function of the full length GP88 which permits its utility in accordance with the present invention.

A "fragment" of GP88 refers to any subset of the molecule that is a shorter peptide retaining the tumorigenic properties of GP88. This corresponds for example but is not limited to regions such as K19T and S14R for mouse GP88, and E19V and A14R (equivalent to murine K19T and S14R, respectively) for human GP88.

A "variant" of GP88 refers to a molecule substantially similar to either the entire peptide or a fragment thereof. Variant peptides may be prepared by direct chemical synthesis of the variant peptide using methods known in the art.

Alternatively, amino acid sequence variants of the peptide can be prepared by modifying the DNA which encodes the synthesized protein or peptide. Such variants include, for example, deletions, insertions, or substitutions of residues within the amino-acid sequence of GP88. Any combination of deletion, insertion, and substitution may also be made to arrive at the final construct, provided the final construct possesses the desired activity. The mutation that will be made in the DNA encoding the variant peptide must not alter the reading frame and preferably will not create complementary regions that could produce secondary mRNA structures. At the genetic level, these variants are prepared by site directed mutagenesis (8) of nucleotides in the DNA encoding the peptide molecule thereby producing DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture. The variant typically exhibits the same qualitative biological activity as the nonvariant peptide.

An "analog" of GP88 protein refers to a non-natural molecule substantially similar to either the entire molecule or a fragment thereof.

A "chemical derivative" contains additional chemical moieties not normally a part of the peptide or protein. Covalent modifications of the peptide are also included within the scope of this invention. Such modifications may be introduced into the molecule by reacting targeted amino-acid residues of the peptide with an organic derivatizing agent that is capable of reacting with selected side chains or terminal amino-acid residues. Most commonly derivatized residues are cysteinyl, histidyl, lysinyl, arginyl, tyrosyl, glutaminyl, asparaginyl and amino terminal residues. Hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl and threonyl residues, methylation of the alpha-amino groups of lysine, histidine, and histidine side chains, acetylation of the N-terminal amine and amidation of the C-terminal carboxylic groups. Such derivatized moieties may improve the solubility, absorption, biological half life and the like. The moieties may also eliminate or

attenuate any undesirable side effect of the protein and the like. In addition, derivatization with bifunctional agents is useful for cross-linking the peptide to water insoluble support matrices or to other macromolecular carriers. Commonly used cross-linking agents include glutaraldehyde, N-hydroxysuccinimide esters, homobifunctional imidoesters, 1,1-bis(-diazoloacetyl)-2-phenylethane, and bifunctional maleimides. Derivatizing agents such as methyl-3-[9p-azidophenyl]dithiopropioimidate yield photoactivatable intermediates that are capable of forming crosslinks in the presence of light. Alternatively, reactive water-insoluble matrices such as cyanogen bromide activated carbohydrates and the reactive substrates described in U.S. Pat. Nos. 3,969,287 and 3,691,016 may be employed for protein immobilization.

Use of the term GP88 antagonist or GP88 "antagonizing agents" herein means any composition that inhibits or blocks GP88 expression, production or secretion, or any composition that inhibits or blocks the biological activity of GP88. This can be achieved by any mode of action such as but not limited to the following:

(A) GP88 antagonizing agents include any reagent or molecule inhibiting GP88 expression or production including but not limited to: (1) antisense GP88 DNA or RNA molecules that inhibit GP88 expression by inhibiting GP88 translation; (2) small inhibitory or "siRNA" that inhibit GP88 expression (3) reagents (hormones, growth factors, small molecules) that inhibit GP88 mRNA and/or protein expression at the transcriptional, translational or post-translational levels; (4) factors, reagents or hormones that inhibit GP88 secretion.

(B) GP88 antagonizing agents also include any reagent or molecule that will inhibit GP88 action or biological activity such as but not limited to: (1) neutralizing antibodies to GP88 that bind the protein and prevent it from exerting its biological activity; (2) antibodies to the GP88 receptor that prevent GP88 from binding to its receptor and from exerting its biological activity; (3) competitive inhibitors of GP88 binding to its receptors; (4) small molecule antagonists; and (5) inhibitors of GP88 signaling pathways.

In one embodiment of the invention, the GP88 antagonizing agents are antisense oligonucleotides to GP88. The antisense oligonucleotides preferably inhibit GP88 expression by inhibiting translation of the GP88 protein. Antisense oligonucleotides may be formed from DNA or RNA. In another embodiment, the GP88 antagonizing agents are small-inhibitory RNA molecules (siRNA or RNAi). siRNA are double-stranded RNA molecules capable of suppressing the expression of a target gene.

GP88 antagonizing agents may comprise small molecules (e.g., reagents, factors or hormones) that inhibit GP88 expression. For example, embodiments of the invention provide small molecules that (1) inhibit GP88 post-translational modification and its secretion, (2) block GP88 activity by competing with GP88 for binding to GP88 cell surface receptors, (3) inhibit the GP88 signal transduction pathway (e.g., biochemical interactions induced by GP88 binding to its receptor on the cell surface), or (4) interfere or inhibit with the GP88 receptor. Small molecules may be synthesized in order to bind to or associate with particular active sites on GP88, GP88 cell surface receptors, or other molecules. Small molecules can also be derived from natural sources and modified to bind to and/or inhibit GP88, GP88 cell surface receptors, or other molecules.

The antibodies of the invention (neutralizing and others) are preferably used as a treatment for breast cancer, other cancers, or other diseases in cells which exhibit an increased

expression of GP88 (e.g., neuroblastoma, glioblastoma, astrocytoma, sarcomas, and cancers of the prostate, blood, cerebrospinal fluid, liver, kidney, breast, head and neck, pharynx, thyroid, pancreas, stomach, colon, colorectal, uterus, cervix, bone, bone marrow, testes, brain, neural tissue, ovary, skin, and lung). By the term "neutralizing" it shall be understood that the antibody has the ability to inhibit or block the normal biological activity of GP88, including GP88's ability to stimulate cell proliferation or to induce tumor growth in experimental animals and in humans. An effective amount of anti-GP88 antibody is administered to an animal, including humans, by various routes. In an alternative embodiment, the anti-GP88 antibody is used as a diagnostic to detect cells which exhibit an altered (increased) expression of GP88 as occurring in diseases such as but not limited to cancers (e.g., breast cancer), and to identify diseased cells whose growth is dependent on GP88 and which will respond to GP88 antagonizing therapy. In yet another embodiment, the anti-GP88 antibody is used to deliver compounds such as cytotoxic factors or antisense oligonucleotides to cells expressing or responsive to GP88. The cytotoxic factors may be attached, linked, or associated with the anti-GP88 antibody.

The antisense oligonucleotides of the invention are also used as a treatment for cancer in cells which exhibit an increased expression of GP88. An effective amount of the antisense oligonucleotide is administered to an animal, including humans, by various routes.

In one embodiment of the invention, GP88 antagonizing agents are used to inhibit or prevent the initiation and/or progression of tumor cells. For example, GP88 antagonizing agents can be used to prevent the occurrence or re-occurrence of breast cancer, other cancers, or other diseases in cells which exhibit an increased expression of GP88 (e.g., neuroblastoma, glioblastoma, astrocytoma, sarcomas, and cancers of the prostate, blood, cerebrospinal fluid, liver, kidney, breast, head and neck, pharynx, thyroid, pancreas, stomach, colon, colorectal, uterus, cervix, bone, bone marrow, testes, brain, neural tissue, ovary, skin, and lung). GP88 antagonizing agents can also be used to restore a normal phenotype to tumor cells overexpressing GP88.

The invention also provides compositions and methods for inducing apoptosis in a tumor cell comprising administering a GP88 antagonist to the cell. Any GP88 antagonist may be used to induce apoptosis (e.g., GP88 antibody or antibody fragment, GP88 antisense nucleic acid, anti-GP88 siRNA, anti-GP88 small molecule, or anti-GP88 receptor antibody). In one embodiment, after administering a GP88 antagonist, the tumor cells undergoing apoptosis are quantified by measuring the level of an apoptotic marker in the tumor cells. For example, elevated levels of Bcl-2 indicate suppression of apoptosis. The apoptotic state of tumor cells can also be determined by measuring cleavage of PARP to its 85 kDa fragment in tumor cells. Apoptosis can be induced in all tumor cell types, including but not limited to, neuroblastoma, glioblastoma, astrocytoma, sarcomas, and cancers of the prostate, blood, cerebrospinal fluid, liver, kidney, breast, head and neck, pharynx, thyroid, pancreas, stomach, colon, colorectal, uterus, cervix, bone, bone marrow, testes, brain, neural tissue, ovary, skin, and lung.

Methods of inducing apoptosis in a tumor comprising co-administering a GP88 antagonist and an anti-estrogen also are provided. In one embodiment, the anti-estrogen is selected from the group consisting of tamoxifen, aromatase inhibitors (e.g., Arimidex®, Femera®), and estrogen-receptor downregulators (e.g., Faslodex®).

The present invention provides methods of determining whether cells are undergoing apoptosis comprising measuring the level of GP88 protein in a first biological sample, measuring the level of GP88 protein in a second biological sample, and determining whether the measured level of GP88 protein in the second biological sample is lower than the level of GP88 protein in the first biological sample by an amount sufficient to indicate the cells are undergoing apoptosis. Such biological samples can be derived from fluids and/or tissues including, but not limited to, prostate, blood, bladder, cerebrospinal fluid, serum, plasma, urine, nipple aspirate, thyroid, head and neck, cervix, liver, kidney, breast, pancreas, stomach, colon, nasopharynx, colorectal, uterus, bone, bone marrow, testes, brain, neural tissue, ovary, skin, and lung tissue.

The present invention also provides methods for targeting GP88 antagonizing reagents to the diseased site by conjugating them to an anti-GP88 antibody or an anti-GP88 receptor antibody.

The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate embodiments of the invention and, together with the description, serve to explain the principles of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1C show the nucleotide (SEQ ID NO: 1) and deduced amino-acid sequence (SEQ ID NO: 2) of mouse GP88. Peptide regions used as antigens to raise anti-GP88 antibodies K19T and S14R are underlined. The region cloned in the antisense orientation in the pCMV4 mammalian expression vector is indicated between brackets.

FIG. 2A shows the nucleotide sequence (SEQ ID NO: 3) of human GP88 cDNA. Indicated between brackets is the region cloned in the antisense orientation into the pcDNA3 mammalian expression system; and

FIG. 2B shows the deduced amino-acid sequence (SEQ ID NO: 4) of human GP88. The E19V region used as antigen to develop anti-human GP88 neutralizing antibody is underlined. It also indicates the region A14R equivalent to the mouse S14R region.

FIG. 3 shows the amino-acid sequence (SEQ ID NO: 2) of mouse GP88 arranged to show the 7 and one-half repeats defined as granulins g, f, B, A, C, D and e (right side). The consensus sequence is shown in SEQ ID NO: 17. This representation shows that the region K19T and S14R used to raise GP88 antibodies for developing anti-GP88 neutralizing antibodies is found between two epithelin/granulin repeats in what is considered a variant region. Indicated on the right hand side is the granulin classification of the repeats according to Bateman et al (6). Granulin B and granulin A are also defined as epithelin 2 and epithelin 1 respectively according to Plowman et al., 1992 (5).

FIG. 4 shows a schematic representation of pCMV4 and a GP88 cDNA clone indicating the restriction sites used to clone GP88 antisense cDNA into the expression vector.

FIG. 5 shows that GP88 inhibits apoptosis in MCF-7 cells in response to tamoxifen. PARP (poly (ADP ribose) polymerase), an apoptosis marker, is cleaved to produce an 85 kDa fragment in the presence of tamoxifen, but not in the presence of GP88 or tamoxifen plus GP88.

FIG. 6 shows that anti-GP88 antibodies induce apoptosis. Tamoxifen-resistant MCF-7 cells are unable to downregulate Bcl-2. In the presence of GP88 antibodies or GP88 antibodies plus tamoxifen, Bcl-2 is downregulated.

FIG. 7 also shows that anti-GP88 antibodies induce apoptosis. O4 cells (overexpressing GP88) are unable to

cleave PARP. In the presence of anti-GP88 antibodies or anti-GP88 antibodies plus tamoxifen, PARP is cleaved, indicating induction of apoptosis.

FIG. 8 also shows that anti-GP88 antibodies induce apoptosis. Tamoxifen-resistant MCF-7 cells are unable to cleave PARP. In the presence of anti-GP88 antibodies or anti-GP88 antibodies plus tamoxifen, PARP is cleaved, indicating induction of apoptosis.

FIG. 9 shows that tamoxifen can induce an increase in tumor volume in GP88 overexpressing cells (O4 cells) in vivo. Thus, treatment with tamoxifen may pose a risk of promoting tumor growth in tumors in which GP88 levels are elevated.

DETAILED DESCRIPTION OF THE INVENTION

Reference will now be made in detail to the presently preferred embodiments of the invention, which, together with the following examples, serve to explain the principles of the invention.

Biological Activity of GP88

The invention relates to GP88 and antitumor and antiviral compositions useful for treating and diagnosing diseases linked to altered (increased) expression of GP88. In addition, this invention is used for treating and diagnosing diseases linked to increased responsiveness to GP88. In accordance with preferred embodiments of the invention, GP88 antagonizing agents (e.g., antibodies, antisense, siRNA, small molecules) can be used to restore sensitivity to the antitumorigenic effects of antiestrogen therapy and induce apoptosis.

Anti-GP88 Antibodies

The invention provides compositions for treating and diagnosing diseases linked to increased expression of GP88 including treatment and diagnosis of diseases linked to increased responsiveness to GP88. The compositions of this invention include anti-GP88 antibodies which neutralize the biological activity of GP88.

The present invention is also directed to an antibody specific for an epitope of GP88 and the use of such antibody to detect the presence or measure the quantity or concentration of GP88 molecule, a functional derivative thereof or a homologue from different animal species in a cell, a cell or tissue extract, culture medium or biological fluid (e.g., whole blood, serum, plasma, lymph, and urine). Moreover, anti-GP88 antibody can be used to target cytotoxic molecules to a specific site.

For use as antigen for development of antibodies, the GP88 protein naturally produced or expressed in recombinant form or functional derivative thereof, preferably having at least 9 amino-acids, is obtained and used to immunize an animal for production of polyclonal or monoclonal antibody. An antibody is said to be capable of binding a molecule if it is capable of reacting with the molecule to thereby bind the molecule to the antibody. The specific reaction is meant to indicate that the antigen will react in a highly selective manner with its corresponding antibody and not with the multitude of other antibodies which may be evoked by other antigens.

The term antibody herein includes but is not limited to human and non-human polyclonal antibodies, human and non-human monoclonal antibodies (mAbs), chimeric anti-

bodies, anti-idiotypic antibodies (anti-IdAb) and humanized antibodies. Polyclonal antibodies are heterogeneous populations of antibody molecules derived either from sera of animals immunized with an antigen or from chicken eggs.

Monoclonal antibodies ("mAbs") are substantially homogeneous populations of antibodies to specific antigens. mAbs may be obtained by methods known to those skilled in the art (e.g., U.S. Pat. No. 4,376,110). Such antibodies may be of any immunological class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing human and non-human antibodies to GP88 may be cultivated in vitro or in vivo. For production of a large amount of mAbs, in vivo is the presently preferred method of production. Briefly, cells from the individual hybridomas are injected intraperitoneally into pristane primed Balb/c mice or Nude mice to produce ascites fluid containing high concentrations of the desired mAbs. mAbs may be purified from such ascites fluids or from culture supernatants using standard chromatography methods well known to those of skill in the art.

Human monoclonal Ab to human GP88 can be prepared by immunizing transgenic mice expressing human immunoglobulin genes. Hybridoma produced by using lymphocytes from these transgenic animals will produce human immunoglobulin instead of mouse immunoglobulin.

Since most monoclonal antibodies are derived from murine source and other non-human sources, their clinical efficiency may be limited due to the immunogenicity of rodent mAbs administered to humans, weak recruitment of effector function and rapid clearance from serum. To circumvent these problems, the antigen-binding properties of murine antibodies can be conferred to human antibodies through a process called humanization. A humanized antibody contains the amino-acid sequences for the 6 complementarity-determining regions (CDRs) of the parent murine mAb which are grafted onto a human antibody framework. The low content of non-human sequences in humanized antibodies (around 5%) has proven effective in both reducing the immunogenicity and prolonging the serum half life in humans. Methods such as the ones using monovalent phage display and combinatorial library strategy for humanization of monoclonal antibodies are now widely applied to the humanization of a variety of antibodies and are known to people skilled in the art. These humanized antibodies and human antibodies developed with transgenic animals as described above are of great therapeutic use for several diseases including but not limited to cancer.

Hybridoma supernatants and sera are screened for the presence of antibody specific for GP88 by any number of immunoassays including dot blots and standard immunoassays (EIA or ELISA) which are well known in the art. Once a supernatant has been identified as having an antibody of interest, it may be further screened by Western blotting to identify the size of the antigen to which the antibody binds. One of ordinary skill in the art will know how to prepare and screen such hybridomas without undue experimentation in order to obtain a desired polyclonal or mAb.

Chimeric antibodies have different portions derived from different animal species. For example, a chimeric antibody might have a variable region from a murine mAb and a human immunoglobulin constant region. Chimeric antibodies and methods for their production are also known to those skilled in the art.

Accordingly, mAbs generated against GP88 may be used to induce human and non-human anti-IdAbs in suitable animals. Spleen cells from such immunized mice are used to produce hybridomas secreting human or non-human anti-Id

mAbs. Further, the anti-Id mAbs can be coupled to a carrier such as Keyhole Limpet Hemocyanin (KLH) or bovine serum albumin (BSA) and used to immunize additional mice. Sera from these mice will contain human or non-human anti-anti-IdAb that have the binding properties of the original mAb specific for a GP88 polypeptide epitope. The anti-Id mAbs thus have their own idiotypic epitopes or idiotypes structurally similar to the epitope being evaluated.

The term antibody is also meant to include both intact molecules as well as fragments thereof such as, for example, Fab and F(ab')₂, which are capable of binding to the antigen. Fab and F(ab')₂ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation and may have less non-specific tissue binding than an intact antibody. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to generate Fab fragments) and pepsin (to generate F(ab')₂ fragments). It will be appreciated that Fab and F(ab')₂ and other fragments of the antibodies useful in the present invention may be used for the detection or quantitation of GP88, and for treatment of pathological states related to GP88 expression, according to the methods disclosed herein for intact antibody molecules.

According to the present invention, antibodies that neutralize GP88 activity in vitro can be used to neutralize GP88 activity in vivo to treat diseases associated with increased GP88 expression or increased responsiveness to GP88. A subject, preferably a human subject, suffering from a disease associated with increased GP88 expression is treated with an antibody to GP88. Such treatment may be performed in conjunction with other anti-cancer or anti-viral therapy. A typical regimen comprises administration of an effective amount of the antibody specific for GP88 administered over a period of one or several weeks and including between about one and six months. The antibody of the present invention may be administered by any means that achieves its intended purpose. For example, administration may be by various routes including but not limited to subcutaneous, intravenous, intradermal, intramuscular, intraperitoneal and oral. Parenteral administration can be by bolus injection or by gradual perfusion over time. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions and emulsions, which may contain auxiliary agents or excipients known in the art. Pharmaceutical compositions such as tablets and capsules can also be prepared according to routine methods. GP88 antagonists can be formulated in any suitable pharmaceutically acceptable carrier (e.g., tablets, pills, injections, infusions, inhalations, transdermal patches, and suppositories). It is understood that the dosage will be dependent upon the age, sex and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment and the nature of the effect desired. The ranges of effective doses provided below are not intended to limit the invention and merely represent preferred dose ranges. However, the most preferred dosage will be tailored to the individual subject as is understood and determinable by one skilled in the art. The total dose required for each treatment may be administered by multiple doses or in a single dose. Effective amounts of antibody are from about 0.01 µg to about 100 mg/kg body weight and preferably from about 10 µg to about 50 mg/kg. Antibody may be administered alone or in conjunction with other therapeutics directed to the same disease.

According to the present invention and concerning the neutralizing antibody, GP88 neutralizing antibodies can be used in all therapeutic cases where it is necessary to inhibit GP88 biological activity, even though there may not necessarily be a change in GP88 expression, including cases

where there is an overexpression of GP88 cell surface receptors and this in turn results in an increased biological activity, or where there is an alteration in GP88 signaling pathways or receptors leading to the fact that the signaling pathways are always "turned on." Neutralizing antibodies to growth factor and to growth factor receptors have been successfully used to inhibit the growth of cells whose proliferation is dependent on this growth factor. This has been the case for IGF-I receptor in human breast carcinoma cells and bombesin for lung cancer. The antibody to GP88 can also be used to deliver compounds such as, but not limited to, cytotoxic reagents such as toxins, oncotoxins, mitotoxins and immunotoxins, or antisense oligonucleotides, in order to specifically target them to cells expressing or responsive to GP88.

One region that allows antigen to develop a neutralizing antibody to GP88 is the 19 amino-acid region defined as K19T in the mouse GP88, and E19V in the human GP88 which is not located within the epithelin/granulin 6 kDa repeats but between these repeats, specifically between granulin A (epithelin 1) and granulin C in what is considered a variant region (see FIG. 3). Without wishing to be bound by theory, it is believed that the region important for the biological activity of GP88 lies outside of the epithelin repeats.

The antibodies or fragments of antibodies useful in the present invention may also be used to quantitatively or qualitatively detect the presence of cells which express the GP88 protein. This can be accomplished by immunofluorescence techniques employing a fluorescently labeled antibody (see below) with fluorescent microscopic, flow cytometric, or fluorometric detection. The reaction of antibodies and polypeptides of the present invention may be detected by immunoassay methods well known in the art.

The antibodies of the present invention may be employed histologically as in light microscopy, immunofluorescence or immunoelectron microscopy, for in situ detection of the GP88 protein in tissues samples, biopsies, and biological fluids. In situ detection may be accomplished by removing a histological specimen from a patient and applying the appropriately labeled antibody of the present invention. The antibody (or fragment) is preferably provided by applying or overlaying the labeled antibody (or fragment) to the biological sample. Through the use of such a procedure, it is possible to determine not only the presence of the GP88 protein but also its distribution in the examined tissue or concentration in a biological fluid. Using the present invention, those of ordinary skill in the art will readily perceive that any wide variety of histological methods (such as staining procedures) can be modified in order to achieve such in situ detection.

Assays for GP88 typically comprise incubating a biological sample such as a biological fluid, a tissue extract, freshly harvested or cultured cells or their culture medium in the presence of a detectably labeled antibody capable of identifying the GP88 protein and detecting the antibody by any of a number of techniques well known in the art.

The biological sample may be treated with a solid phase support or carrier such as nitrocellulose or other solid support capable of immobilizing cells or cell particles or soluble proteins. The support may then be washed followed by treatment with the detectably labeled anti-GP88 antibody. This is followed by wash of the support to remove unbound antibody. The amount of bound label on said support may then be detected by conventional means. The term solid phase support refers to any support capable of binding

antigen or antibodies such as but not limited to glass, polystyrene polypropylene, nylon, modified cellulose, or polyacrylamide.

The binding activity of a given lot of antibody to the GP88 protein may be determined according to well known methods. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation.

Detection of the GP88 protein or functional derivative thereof and of a specific antibody for the protein may be accomplished by a variety of immunoassays well known in the art such as enzyme linked immunoassays (EIA) or radioimmunoassays (RIA). Such assays are well known in the art and one of skill will readily know how to carry out such assays using the anti-GP88 antibodies and GP88 protein of the present invention.

Such immunoassays are useful to detect and quantitate GP88 protein in serum or other biological fluid as well as in tissues, cells, cell extracts, or biopsies. In a preferred embodiment, the concentration of GP88 is measured in a tissue specimen as a means for diagnosing cancer or other disease associated with increased expression of GP88. In another preferred embodiment, the concentration of GP88 in a biological fluid sample is used to determine if a patient is likely to be responsive, or is responding to, anti-tumorigenic therapy.

The presence of certain types of cancers (e.g., breast cancer) and the degree of malignancy are said to be "proportional" to an increase in the level of the GP88 protein. The term "proportional" as used herein is not intended to be limited to a linear or constant relationship between the level of protein and the malignant properties of the cancer. The term "proportional" as used herein, is intended to indicate that an increased level of GP88 protein is related to appearance, recurrence or display of malignant properties of a cancer or other disease associated with increased expression of GP88 at ranges of concentration of the protein that can be readily determined by one skilled in the art.

Another embodiment of the invention relates to evaluating the efficacy of anti-cancer or anti-viral drug or agent by measuring the ability of the drug or agent to inhibit the expression or production of GP88. The antibodies of the present invention are useful in a method for evaluating anti-cancer or anti-viral drugs in that they can be employed to determine the amount of the GP88 protein in one of the above-mentioned immunoassays. Alternatively, the amount of the GP88 protein produced is measured by bioassay (cell proliferation assay) as described herein. The bioassay and immunoassay can be used in combination for a more precise assessment.

An additional embodiment is directed to an assay for diagnosing cancers or other diseases associated with an increase in GP88 expression based on measuring in a tissue or biological fluid the amount of mRNA sequences present that encode GP88 or a functional derivative thereof, preferably using an RNA-DNA hybridization assay. The presence of certain cancers and the degree of malignancy is proportional to the amount of such mRNA present. For such assays the source of mRNA will be biopsies and surrounding tissues. The preferred technique for measuring the amount of mRNA is a hybridization assay using DNA of complementarity base sequence.

Another related embodiment is directed to an assay for diagnosing cancers or other diseases associated with an increase in GP88 responsiveness based on measuring on a tissue biopsy whether treatment with anti-GP88 neutralizing antibody will inhibit its growth or other biological activity.

Another related embodiment is a method for measuring the efficacy of anti-cancer or anti-viral drug or agent which comprises the steps of measuring the agent's effect on inhibiting the expression of mRNA for GP88. Similarly such method can be used to identify or evaluate the efficacy of GP88 antagonizing agents by measuring the ability of said agent to inhibit the production of GP88 mRNA.

Nucleic acid detection assays, especially hybridization assays, can be based on any characteristic of the nucleic acid molecule such as its size, sequence, or susceptibility to digestion by restriction endonucleases. The sensitivity of such assays can be increased by altering the manner in which detection is reported or signaled to the observer. A wide variety of labels have been extensively developed and used by those of ordinary skill in the art, including enzymatic, radioisotopic, fluorescent, chemical labels and modified bases.

One method for overcoming the sensitivity limitation of a nucleic acid for detection is to selectively amplify the nucleic acid prior to performing the assay. This method has been referred as the "polymerase chain reaction" or PCR (U.S. Pat. No. 4,683,202 and 4,582,788). The PCR reaction provides a method for selectively increasing the concentration of a particular nucleic acid sequence even when that sequence has not been previously purified and is present only in a single copy in a particular sample.

Restoration of Sensitivity To Antiestrogen Therapy And Cytotoxic Therapy

Tumors progress through various stages of growth and maturation leading to increased mobility of the tumor through the body. Therefore, tumor cells can be targeted at several stages during the progression of tumor growth and maturation. For example, GP88 antagonists inhibit tumor cell growth and proliferation. For example, GP88 induces expression of cyclin D1, a cell cycle regulatory protein involved in the growth and proliferation of tumor cells. As shown in FIG. 11, anti-GP88 antibodies inhibit cyclin D1 expression. Lanes 1 and 2 of FIG. 11 show that anti-GP88 antibody 5B4 inhibits the expression of cyclin D1. Lanes 3 and 4 show that GP88 stimulates cyclin D1 expression. Lanes 5 and 6 demonstrate that anti-GP88 antibody 5B4 blocks induction of cyclin D1 by GP88.

A preferred embodiment of the invention provides a method for restoring sensitivity of tumor cells to antiestrogen therapy, comprising contacting a tumor cell that is nonsensitive to antitumorigenic effects of antiestrogen therapy with a GP88 antagonist in an amount sufficient to restore sensitivity to the antitumorigenic effects of antiestrogen therapy. In one embodiment, the term "restoring sensitivity" refers to increasing the responsiveness of a treated tumor cell to the antitumorigenic effects of antiestrogen therapy. For example, a tumor cell that does not respond to antiestrogen therapy (i.e., continues to grown in an uncontrolled manner), responds to antiestrogen therapy (i.e., is growth inhibited, undergoes apoptosis, etc.) after being contacted with a GP88 antagonist.

The GP88 antagonists or GP88 antagonizing agents include, but are not limited to, anti-GP88 antibodies or antibody fragments, anti-GP88 receptor antibodies, anti-GP88 small molecules, anti-GP88 antisense nucleic acids, and siRNA. GP88 antagonists can prevent tumor formation and growth of any tumor or cancer type, including but not limited to, prostate, head and neck, nasopharynx, thyroid,

pancreas, bladder, cervix, colorectal, blood, liver, kidney, breast, bone, bone marrow, testes, ovaries, brain, neural, colon, and lung tumors.

In one embodiment, sensitivity of tumor cells to effects of antiestrogen therapy can be increased by inducing apoptosis. Apoptosis can be induced by contacting an anti-estrogen and GP88 antagonist with a tumor cell. Anti-estrogen therapy relates to administration of anti-estrogens for the purpose of preventing or treating tumor growth. As discussed above, anti-estrogens such as tamoxifen inhibit tumor growth, in part, by inducing apoptosis. However, as shown in FIG. 9, tamoxifen alone may actually stimulate tumor growth in vivo in cells overexpressing GP88. Thus, treatment of patients having high levels of GP88 with anti-estrogens alone may be counterproductive.

Treatment of tumors overexpressing GP88 with both anti-estrogens and anti-GP88 antibodies can induce apoptosis and eliminate the potential growth stimulating properties of anti-estrogen treatment on GP88 overexpressing cells. Examples of anti-estrogens include tamoxifen citrate ("tamoxifen"), a nonsteroidal anti-estrogen commonly prescribed to patients suffering from breast cancer that has demonstrated potent anti-estrogenic and antineoplastic properties. (See U.S. Pat. No. 4,536,516), raloxifene, aromatase inhibitors (e.g., Arimidex® (anastrozole), Femera®, letrozole), and estrogen receptor down-regulators (e.g., Faslodex®).

Tamoxifen-induced apoptosis is blocked in tumor cells overexpressing GP88. FIG. 5 is a western blot measuring the level of the PARP cleavage product (85 kDa band) in MCF-7 breast carcinoma cells treated with tamoxifen, tamoxifen+GP88, tamoxifen+estradiol, GP88, and a control. As shown in FIG. 5, tamoxifen treatment induced apoptosis of MCF-7 cells. The 85 kDa PARP cleavage product was significantly increased in the "Tamoxifen" labeled lane, indicating apoptosis. Tamoxifen-induced apoptosis was blocked by treatment with estradiol, a compound known to block tamoxifen-induced apoptosis, as shown by the decrease in the 85 kDa band in the lane labeled "Tamoxifen+estradiol." Tamoxifen-induced apoptosis was blocked by treatment with GP88 to the same extent as treatment by estradiol. The lane labeled "Tamoxifen+GP88" shows a significant decrease in the PARP cleavage product compared to the "Tamoxifen" lane. FIG. 5 shows that GP88 blocks tamoxifen-induced apoptosis in tumor cells.

Additional embodiments of the invention are directed to compositions and methods of inducing apoptosis. Apoptosis, also known as programmed cell death, is an essential component of the growth regulatory mechanism of a cell. For example, apoptosis is a mechanism for ridding the body of damaged cells (e.g., viral infected cells, cells with DNA damage). Cells undergoing apoptosis are typically smaller than normal cells and have highly condensed nuclei. Apoptotic cells are marked for clearance by the immune system.

Deficient regulation of apoptosis can cause uncontrolled cell growth and tumorigenicity. Oncogenes can be activated by DNA damage. Under normal apoptotic conditions, the body would clear the damaged cell. Under abnormal conditions, apoptosis does not occur and the damaged cell grows and proliferates leading to tumorigenesis. For example, p53 normally functions to induce apoptosis. However, the oncogenic form of p53 blocks induction of apoptosis leading to uncontrolled cell growth. Unregulated cell survival contributes to diseases such as cancer, autoimmune diseases, and inflammatory diseases. As discussed above, the Bcl-2 gene encodes a protein capable of blocking

apoptosis. Overexpression of Bcl-2 indicates that apoptosis is blocked and the cell will not undergo programmed cell death.

The present invention provides methods and compositions for restoring sensitivity to the antitumorigenic effects of cytotoxic or chemotherapeutic agents. For example, GP88 antagonists (e.g., anti-GP88 antibodies, GP88 antisense nucleic acids, siRNA, and small molecules, etc.) can be administered to patients who develop resistance to the antitumorigenic effects of cytotoxic agents (e.g., Altretamine, Bleomycin, Busulphan, Calcium Folate, Capecitabine, Carboplatin, Carmustine, Chlorambucil, Cisplatin, Cladribine, Crisantaspase, Cyclophosphamide, Cytarabine, Dacarbazine, Dactinomycin, Daunorubicin, Docetaxel, Doxorubicin, Epirubicin, Etoposide, Fludarabine, Fluorouracil, Gemcitabine, Hydroxyurea, Idarubicin, Ifosfamide, Irinotecan, Liposomal doxorubicin, Lomustine, Melphalan, Mercaptopurine, Methotrexate, Mitomycin, Mitoxantrone, Oxaliplatin, Paclitaxel, Pentostatin, Procarbazine, Raltitrexed, Streptozocin, Tegafur-uracil, Temozolomide, Thiotepa, Tioguanine/Thioguanine, Topotecan, Treosulfan, Vinblastine, Vincristine, Vindesine, and Vinorelbine). Sensitivity and responsiveness to cytotoxic and/or chemotherapeutic agents and combinations thereof can be restored.

In another embodiment of the invention, GP88 antagonists can be co-administered with an antiestrogen and/or a cytotoxic compound to a tumor cell. For example, an anti-GP88 antibody can be co-administered to a tumor cell with tamoxifen to restore sensitivity to the antitumorigenic effects of tamoxifen or to prevent development of tamoxifen resistance. In another embodiment, the GP88 antagonist can be administered sequentially with an antiestrogen and/or a cytotoxic compound. Thus, GP88 antagonist can be administered either before or after administration of the antiestrogen or cytotoxic compound.

The present invention also provides methods of inducing apoptosis comprising administering a GP88 antagonist to a tumor cell. Apoptosis can be induced in all tumor cell types including, but not limited to, neuroblastoma, glioblastoma, astrocytoma, sarcomas, and cancers of the prostate, blood, cerebrospinal fluid, liver, kidney, breast, head and neck, pharynx, thyroid, pancreas, stomach, colon, colorectal, uterus, cervix, bone, bone marrow, testes, brain, neural tissue, ovary, skin, and lung. Apoptosis can be detected in tumor cells by a variety of techniques including, but not limited to for example, detection of Bcl-2 levels or detection of the PARP cleavage product, DNA ladder, and production of apoptotic cells. PARP or poly(ADP-ribose) polymerase is a nuclear binding protein that detects DNA strand breaks and participates in DNA repair. Cleavage of PARP is a hallmark of caspases-dependent apoptosis. When apoptosis takes place, the 116 kDa intact PARP is cleaved in two fragments, 85 kDa and 25 kDa. Detection of intact and cleaved forms of PARP by Western Blot analysis with anti-PARP antibody has been established as an apoptosis assay. Duriez et al., *Biochem Cell Biol.* 75: 337-49, 1997.

Apoptosis is controlled by the ratio of apoptotic to anti-apoptotic factors, particularly Bcl-2, bcl-xl and bax. Previous reports have suggested that Bcl-2 expression was down-regulated by tamoxifen treatment leading to activation of apoptosis in MCF-7 cells and in tissues from patients treated with tamoxifen. Zhang et al., *Clin. Cancer Res.* 5: 2971-7, 1999; Cameron et al., *Eur. J. Cancer* 36: 845-51, 2000. Alteration of Bcl-2 expression levels changes the bax:Bcl-2 ratio and alters susceptibility to apoptosis. O4 cells are MCF-7 cells transformed with a GP88 expression construct to overexpress GP88. Serrero et al., *Proc. Natl. Acad. Sci.*

U.S.A. 97: 3993-8, 2000. As shown in FIG. 6, tamoxifen induced down-regulation of Bcl-2 transcript in MCF-7 cells at all concentrations tested indicating that tamoxifen induces apoptosis. In contrast, tamoxifen failed to down regulate Bcl-2 in O4 cells even at elevated doses.

Tamoxifen-resistant MCF-7 cells are unable to down-regulate Bcl-2 as shown in FIG. 6. However, the addition of anti-GP88 antibodies to tamoxifen-resistant MCF-7 cells restores apoptosis as indicated by the down-regulation of Bcl-2. (FIG. 6). Thus, GP88 antagonists restore the ability of tamoxifen to induce apoptosis in tamoxifen-resistant cells.

Anti-GP88 antibodies also induce apoptosis in O4 cells (GP88-overexpressing, tamoxifen-resistant MCF-7 cells). As shown in FIG. 7, the PARP cleavage product is increased in O4 cells treated with 5B4 antibody (5B4) or 5B4 plus tamoxifen (5B4+T) indicating that treatment of the cells with anti-GP88 antibody induced apoptosis.

Treatment of tamoxifen resistant MCF-7 cells with anti-GP88 antibodies also induces apoptosis. As shown in FIG. 8, addition of anti-GP88 antibody 5B4 alone or in combination with tamoxifen results in cleavage of PARP while treatment with tamoxifen alone does not cleave PARP.

FIG. 9 shows the effect of tamoxifen on the growth of MCF-7 cells and O4 cells in ovariectomized nude mice. Athymic ovariectomized female nude mice were implanted with an estradiol pellet one day before being injected (S.C) with either MCF-7 cells or O4 cells. After 10 days when the tumors were visible, the mice received either a placebo pellet or tamoxifen pellet. Tumor growth was monitored for 45 days. At the end of the experiments, mice were euthanized and the tumors were excised and evaluated. As shown in FIG. 9, tumor volume in mice with tumors induced by O4 cells (GP88 overexpressing cells) and treated with tamoxifen increased from 50 mm³ to about 225 mm³ after 45 days. In contrast, tumor volume in mice with tumor induced by O4 cells and untreated with tamoxifen increased from 75 mm³ to 150 mm³ in 45 days. Tamoxifen treatment of tumors overexpressing GP88 increased tumor volume more than 4 times while tumor volume of untreated GP88 overexpressing tumors increased by 2 times.

Thus, GP88 antagonizing agents (e.g., anti-GP88 antibodies, GP88 antisense nucleic acids, siRNA, anti-GP88 small molecules, etc.) can be administered to tumor cells and restore the antitumorigenic effects of antiestrogen therapy, for example, by inducing apoptosis.

GP88 antibodies suitable for restoring sensitivity to antiestrogen therapy and cytotoxic therapy, and in other preferred compositions methods of the invention (e.g., inducing apoptosis, etc.) have been deposited with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Va. 20110-2209, and may be produced from hybridoma cell lines, including, but not limited to, 6B3 hybridoma cell line (ATCC Accession Number PTA-5262), 6B2 hybridoma cell line (ATCC Accession Number PTA-5261), 6C12 hybridoma cell line (ATCC Accession Number PTA-5597), 5B4 hybridoma cell line (ATCC Accession Number PTA-5260), 5G6 hybridoma cell line (ATCC Accession Number PTA-5595), 4D1 hybridoma cell line (ATCC Accession Number PTA-5593), 3F8 hybridoma cell line (ATCC Accession Number PTA-5591), 3F5 hybridoma cell line (ATCC Accession Number PTA-5259), 3F4 hybridoma cell line (ATCC Accession Number PTA-5590), 3G2 (ATCC Accession Number PTA-5592), and 2A5 hybridoma cell line (ATCC Accession Number PTA-5589).

In another embodiment of the invention, anti-GP88 receptor antibodies, including antibodies produced from hybridoma cell lines including, but not limited to, 6G8 hybridoma

cell line (ATCC Accession Number PTA-5263) and 5A8 hybridoma cell line (ATCC Accession Number PTA-5594) can be used to restore sensitivity to the antitumorigenic effects of antiestrogen therapy and cytotoxic therapy and induce apoptosis in tumor cells.

Each of these hybridoma cell lines have been deposited with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Va. 20110-2209. 3F5 hybridoma cell line (ATCC Accession Number PTA-5259), 6B3 hybridoma cell line (ATCC Accession Number PTA-5262), 6B2 hybridoma cell line (ATCC Accession Number PTA-5261), 6G8 hybridoma cell line (ATCC Accession Number PTA-5263) and 5B4 hybridoma cell line (ATCC Accession Number PTA-5260) were deposited on Jun. 12, 2003; 5A8 hybridoma cell line (ATCC Accession Number PTA-5594), 5G6 hybridoma cell line (ATCC Accession Number PTA-5595), 4D1 hybridoma cell line (ATCC Accession Number PTA-5593), 3F8 hybridoma cell line (ATCC Accession Number PTA-5591), 3F4 hybridoma cell line (ATCC Accession Number PTA-5590), 3G2 (ATCC Accession Number PTA-5592), 6C12 hybridoma cell line (ATCC Accession Number PTA-5597), and 2A5 hybridoma cell line (ATCC Accession Number PTA-5589) were deposited on Oct. 17, 2003.

Anti-GP88 antibodies and anti-GP88 receptor antibodies (collectively "GP88 antagonist antibodies") can be provided to cells both in vitro and in vivo. For in vitro applications, GP88 antagonist antibodies can be added to cell culture medium at concentrations typically ranging from 0.01 ng to about 100 mg/ml of cell culture media and preferably from about 10 ng to about 50 mg/ml. Antibody may be administered alone or in conjunction with other therapeutics directed to the same disease. Cells can also be transfected with DNA or RNA encoding GP88 antagonist antibodies or antibody fragments or vectors containing such DNA or RNA sequences. Transfected cells can be induced to make GP88 antagonist antibodies or antibody fragments using any suitable technique (e.g., inducible promoter, and multiple plasmid copies).

GP88 antagonist antibody compositions can also be administered to cells using ex vivo techniques. Tumorigenic or normal cells can be removed from a subject (e.g., human or other mammal) and grown in culture. The cells can then be transfected with DNA or RNA encoding GP88 antagonist antibodies and induced to produce GP88 antagonist antibodies. The transfected cells can then be re-introduced into the subject to produce GP88 antagonist antibodies or antibody fragments and inhibit the activity of GP88, reduce tumor cell proliferation, and reduce tumor volume.

For in vivo applications, GP88 antagonist antibody compositions can be provided to a subject by a variety of administration routes and dosage forms. A subject suffering from disease associated with increased GP88 expression may be treated with a GP88 antagonist antibody or fragment. Alternatively, a subject's cells are transfected with a polynucleotide encoding a GP88 antagonist antibody or fragment. A typical regimen comprises administering of an effective amount of the GP88 antagonist antibody over a period of one week to about six months.

The GP88 antagonists of the present invention may be administered by various routes, including, but not limited to, subcutaneous, intravenous, intradermal, intramuscular, intraperitoneal and oral. Parenteral administration can be by bolus injection or by gradual perfusion over time. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions and emulsions, which may contain auxiliary agents or excipients known in the art.

Pharmaceutical compositions such as tablets and capsules can also be prepared. Pharmaceutically acceptable carriers (e.g., tablets, pills, injections, infusions, inhalations, transdermal patches, and suppositories) can be used to administer GP88 antagonists to a patient. The pharmaceutical compositions of the invention comprise GP88 antagonists and can include antiestrogens and/or cytotoxic compounds.

It is understood that the dosage will be dependent upon the age, sex and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment and the nature of the effect desired. The ranges of effective doses provided below are not intended to limit the invention and merely represent illustrative dose ranges. However, the most preferred dosage will be tailored to the individual subject as is understood and determinable by one of ordinary skill in the art given the teachings herein. The total dose required for each treatment may be administered by multiple doses or in a single dose. Effective amounts of antibody are typically from about 0.01 µg to about 100 mg/kg body weight and preferably from about 10 µg to about 50 mg/kg. Antibody may be administered alone or in conjunction with other therapeutics directed to the same disease.

The present invention also provides methods for determining responsiveness to GP88 antagonist treatment comprising measuring the level of GP88 protein in a first biological sample; measuring the level of GP88 protein in a second biological sample; and determining whether the measured level of GP88 protein in the second biological sample is lower than the level of GP88 protein in the first biological sample by an amount sufficient to indicate the cells are responding to GP88 antagonist treatment. The term "responding to GP88 antagonist treatment" refers to preventing, suppressing, and/or decreasing cell growth or tumor cell growth.

The level of GP88 in a first or initial biological sample can be measured and compared to the level of GP88 in a second tumor biological sample taken at a different time. For example, biological samples or biopsies can be taken at regular intervals and the measured concentration of GP88 in subsequent samples can be compared to the GP88 level in the initial sample. A decrease in the level of GP88 in the tumor samples over time is indicative that the cells responding to GP88 antagonist treatment.

GP88 Expression Inhibitors

This invention also provides GP88 expression inhibitors (e.g., antisense components, and siRNA). The term antisense component corresponds to an RNA sequence as well as a DNA sequence coding therefor, which is sufficiently complementary to a particular mRNA molecule, for which the antisense RNA is specific, to cause molecular hybridization between the antisense RNA and the mRNA such that translation of the mRNA is inhibited. Such hybridization can occur under in vivo conditions. The action of the antisense RNA results in specific inhibition of gene expression in the cells. For example, antisense components can block translation of an mRNA and/or block or prevent splicing of mRNA. Antisense molecules can also be directed to bind to introns which are less conserved between species resulting in greater specificity (e.g., inhibiting expression of a gene product of one species but not its homologue in another species).

siRNA are double-stranded (ds) RNA molecules that inhibit gene expression by forming a complex with a target nucleic acid. One strand, called the sense strand is complementary to the target nucleic acid (e.g., messenger RNA

("mRNA")) while the second strand is complementary to the sense strand. Fire et al., *Nature*, 391(6669):806-811 (1998). siRNA or RNAi can be used to inhibit gene expression in a variety of species (e.g., *C. elegans* Id. and *Drosophila* (Kennerdell and Carthew, *Cell* (1998) 95:1017-1026; Misquitta and Patterson, *PNAS* (1999) 96:1451-1456)). In one embodiment, dsRNA used as siRNA can be generated by transcription in vivo. Alternatively, dsRNA can be generated in vitro using the polymerase chain reaction (PCR) or other techniques (e.g., Promega Large Scale RNA Production System (Madison, Wis.)) according to standard and other protocols. In another embodiment, complementary sense and antisense RNA strands can be derived from the sequence of the target gene (e.g., GP88) and can be synthesized. The resulting sense and antisense RNAs can be annealed in a buffer and administered to an animal or used in cell culture experiments. See e.g., Timmons and Fire, *Nature* (1998) 395:854; Montgomery et al., *PNAS* (1998) 95:15502-15507; Tabara et al., *Science* (1998) 282:430-431.

The double-stranded siRNA forms a complex with and cleaves the target mRNA, resulting in destruction of the target RNA. Id. The double-stranded siRNA is incorporated into a complex called RNA-induced silencing complex or RISC. Kim et al., *J. Korean Med Sci* 18:309-18 (2003). RNA helicase catalyzes the destruction of one of the strands of the siRNA activating the RISC complex resulting in the targeting and destruction of the target RNA molecule. Id. siRNA have high specificity for their target genes and can inhibit gene expression by 90% in most genes. Id.

Preferably, siRNA directed to GP88 interfere with the function of GP88 mRNA. These may be directed against any portion of the GP88, preferably of at least about 20 nucleotides in length. siRNA directed against GP88, in accordance with the invention, are capable of inhibiting tumor cell growth, preventing tumor cell growth, inducing tumor regression, and inducing apoptosis.

siRNA directed against GP88 can be targeted against any suitable sequence. siRNA can be made to target any portion of the GP88 nucleotide sequence. After binding to a portion of the GP88 nucleotide sequence, the GP88 nucleic acid is marked for destruction. In one embodiment, the siRNA is generated from a single stranded oligonucleotide having the following sequence: 5' AGGTTGATGCCCACTGCTCTG 3' (SEQ ID NO: 5) (siRNA Sequence 1). siRNA Sequence 1 targets the human GP88 nucleotide region beginning at nucleotide position 203 (FIG. 2A). siRNA targeting the human GP88 sequence beginning at position 223 of GP88 has the following sequence: GAGCAGUGGGCAUCAAC-CUGG (SEQ ID NO: 6) (siRNA Sequence 2). siRNA Sequence 3 (5' AGATCAGGTAACAACCTCCGTG 3') (SEQ ID NO: 7) targets the human GP88 sequence beginning at nucleotide 342. siRNA Sequence 4 (5' GGACACTTCTGC-CATGATAAC 3') (SEQ ID NO: 8) targets the human GP88 sequence beginning at nucleotide 1569.

In one embodiment, the siRNA sequences can be made into double stranded siRNA sequences according to methods well known in the art. It is understood that siRNA can be generated to target any suitable region of the GP88 nucleotide sequence from a variety of species (human, mouse, porcine, canine, etc.). Nucleotide substitutions, deletions, reversions, and mutations can be introduced into the siRNA sequences without altering the function of the siRNA to target the GP88 nucleic acid (e.g., mRNA) for destruction.

Transfection of tumor cells with DNA antisense or siRNA to the GP88 cDNA inhibits endogenous GP88 expression and inhibits tumorigenicity of the transfected cells. This antisense DNA should have sufficient complementarity,

about 18-30 nucleotides in length, to the GP88 gene so that the antisense RNA can hybridize to the GP88 gene (or mRNA) and inhibit GP88 gene expression regardless of whether the action is at the level of splicing, transcription, or translation. The degree of inhibition is readily discernible to one skilled in the art without undue experimentation given the teachings herein and preferably is sufficient to inhibit the growth of cells whose proliferation is dependent on the expression of GP88. One of ordinary skill in the art will recognize that the antisense RNA approach is but a number of mechanisms which can be employed to block specific gene expression.

The antisense components of the present invention may be hybridizable to any of several portions of the target GP88 cDNA, including the coding sequence, 3' or 5' untranslated regions, or other intronic sequences, or to GP88 mRNA. As is readily discernible by one of ordinary skill in the art, the minimal amount of homology required by the present invention is that sufficient to result in hybridization to the GP88 DNA or mRNA and in inhibition of transcription of the DNA, or translation or function of the mRNA, preferably without affecting the function of other mRNA molecules and the expression of other unrelated genes.

Antisense RNA may be delivered to a cell by transformation or transfection via a vector, including retroviral vectors and plasmids, into which has been placed DNA encoding the antisense RNA with the appropriate regulatory sequences including a promoter to result in expression of the antisense RNA in a host cell. Stable transfection of various antisense expression vectors containing GP88 cDNA fragments in the antisense orientation have been performed. One can also deliver antisense components to cells using a retroviral vector. Delivery can also be achieved by, for example, liposomes or nucleofection.

For purposes of in vivo therapy, the currently preferred method is to use antisense oligonucleotides, instead of performing stable transfection of an antisense cDNA fragment constructed into an expression vector. Antisense oligonucleotides having a size of 15-30 bases in length and with sequences hybridizable to any of several portions of the target GP88 cDNA, including the coding sequence, 3' or 5' untranslated regions, or other intronic sequences, or to GP88 mRNA are preferred. Sequences for the antisense oligonucleotides to GP88 are preferably selected as being the ones that have the most potent antisense effects. Factors that govern a target site for the antisense oligonucleotide sequence are related to the length of the oligonucleotide, binding affinity, and accessibility of the target sequence. Sequences may be screened in vitro for potency of their antisense activity by measuring inhibition of GP88 protein translation and GP88 related phenotype, e.g., inhibition of cell proliferation in cells in culture. In general, it is known that most regions of the RNA (5' and 3' untranslated regions, AUG initiation, coding, splice junctions and introns) can be targeted using antisense oligonucleotides.

The preferred GP88 antisense oligonucleotides are those oligonucleotides which are stable, have a high resilience to nucleases (enzymes that could potentially degrade oligonucleotides), possess suitable pharmacokinetics to allow them to traffic to disease tissue at non-toxic doses, and have the ability to cross through plasma membranes.

Phosphorothioate antisense oligonucleotides may be used. Modifications of the phosphodiester linkage as well as of the heterocycle or the sugar may provide an increase in efficiency. With respect to modification of the phosphodiester linkage, phosphorothioate may be used. An N3'-P5' phosphoramidate linkage has been described as stabilizing

oligonucleotides to nucleases and increasing the binding to RNA. Peptide nucleic acid (PNA) linkage is a complete replacement of the ribose and phosphodiester backbone and is stable to nucleases, increases the binding affinity to RNA, and does not allow cleavage by RNase H. Its basic structure is also amenable to modifications that may allow its optimization as an antisense component. With respect to modifications of the heterocycle, certain heterocycle modifications have proven to augment antisense effects without interfering with RNase H activity. An example of such modification is C-5 thiazole modification. Modification of the sugar may also be considered. 2'-O-propyl and 2'-methoxyethoxy ribose modifications stabilize oligonucleotides to nucleases in cell culture and in vivo. Cell culture and in vivo tumor experiments using these types of oligonucleotides targeted to c-raf-1 resulted in enhanced potency.

The delivery route will be the one that provides the best antisense effect as measured according to the criteria described above. In vitro cell culture assays and in vivo tumor growth assays using antisense oligonucleotides have shown that delivery mediated by cationic liposomes, by retroviral vectors and direct delivery are efficient. Another possible delivery mode is targeting using antibody to cell surface markers for the tumor cells. Antibody to GP88 or to its receptor may serve this purpose.

Recombinant GP88

The present invention is also directed to DNA expression systems for expressing a recombinant GP88 polypeptide or a functional derivative thereof substantially free of other mammalian DNA sequences. Such DNA may be double or single stranded. The DNA sequence should preferably have about 20 or more nucleotides to allow hybridization to another polynucleotide. In order to achieve higher specificity of hybridization, characterized by the absence of hybridization to sequences other than those encoding the GP88 protein or a homologue or functional derivative thereof, a length of at least 50 nucleotides is preferred.

The present invention is also directed to the above DNA molecules, expressible vehicles or vectors as well as hosts transfected or transformed with the vehicles and capable of expressing the polypeptide. Such hosts may be prokaryotic, preferably bacteria, or eukaryotic, preferably yeast or mammalian cells. A preferred vector system includes baculovirus expressed in insect cells. The DNA can be incorporated into host organisms by transformation, transduction, transfection, infection or related processes known in the art. In addition to DNA and mRNA sequences encoding the GP88 polypeptide, the invention also provides methods for expression of the nucleic acid sequence. Further, the genetic sequences and oligonucleotides allow identification and cloning of additional polypeptides having sequence homology to the polypeptide GP88 described here.

An expression vector is a vector which (due to the presence of appropriate transcriptional and/or translational control sequences) is capable of expressing a DNA (or cDNA) molecule which has been cloned into the vector and thereby produces a polypeptide or protein. Expression of the cloned sequence occurs when the expression vector is introduced into an appropriate host cell. If a prokaryotic expression vector is employed, then the appropriate host cell would be any prokaryotic cell capable of expressing the cloned sequence. Similarly, if an eukaryotic expression system is employed, then the appropriate host cell would be any eukaryotic cell capable of expressing the cloned sequence.

Baculovirus vector, for example, can be used to clone GP88 cDNA and subsequently express the cDNA in insect cells.

A DNA sequence encoding GP88 polypeptide or its functional derivatives may be recombined with vector DNA in accordance with conventional techniques including blunt-ended or staggered ended termini for ligation, restriction enzyme digestion to provide appropriate termini, filling in cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and ligation with proper enzyme ligases. Techniques for such manipulations are discussed in (35).

A nucleic acid molecule is capable of expressing a polypeptide if it contains nucleotide sequences which contain transcriptional and translational regulatory information and such sequences are operably linked to nucleotide sequences which encode the polypeptide. An operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequence sought to be expressed are connected in such a way as to permit gene expression. The precise nature of the regulatory regions needed for gene expression may vary from organism to organism but shall in general include a promoter region, which in prokaryotes contains both the promoter (which directs the initiation of RNA transcription) as well as the DNA sequences which when transcribed into RNA will signal the initiation of protein synthesis. Such regions will normally include those 5' non-coding sequences involved with the initiation of transcription, translation such as the TATA box, capping sequence, CAAT sequence and the like.

If desired, the 3' non-coding region to the gene sequence encoding the protein may be obtained by described methods (screening appropriate cDNA library or PCR amplification). This region may be retained for the presence of transcriptional termination regulatory sequences such as termination and polyadenylation. Thus, by retaining the 3' region naturally contiguous to the DNA sequence coding for the protein, the transcriptional termination signals may be provided. Where the transcription termination signals are not provided or satisfactorily functional in the expression host cells, then a 3' region from another gene may be substituted.

Two DNA sequences such as a promoter region sequence and GP88 encoding sequence are said to be operably linked if the nature of the linkage between the sequences does not result in the introduction of a frame-shift mutation or interfere with the ability of the promoter sequence to direct transcription of the polypeptide gene sequence. The promoter sequences may be prokaryotic, eukaryotic or viral. Suitable promoters are inducible, repressible or constitutive.

Eukaryotic promoters include, but are not limited to, the promoter for the mouse methallothionein I gene, the TK promoter of Herpes Virus, the gene gal4 promoter, the SV40 early promoter, the mouse mammary tumor virus (MMTV) promoter, and the cytomegalovirus (CMV) promoter. Strong promoters are preferred. Examples of such promoters are those which recognize the T3, SP6 and T7 polymerases, the PL promoter of bacteriophage lambda, the recA promoter, the promoter of the mouse methallothionein I gene, the SV40 promoter and the CMV promoter.

It is to be understood that application of the teachings of the present invention to a specific problem or environment will be within the capability of one having ordinary skill in the art in light of the teachings contained herein. The present invention is more fully illustrated by the following non-limiting examples.

EXAMPLE 1

GP88 Antagonists Induce Apoptosis In Tamoxifen-Treated Cells

GP88 overexpression blocks down-regulation of Bcl-2 mRNA transcript in response to tamoxifen treatment. MCF-7 cells and MCF-7 cells overexpressing GP88 (O4 cells) were treated with 0 to 2 μ m tamoxifen. FIG. 6. 5 μ g of total RNA was reverse transcribed into single strand cDNA by Super Script II (BRL, Gaithersburg, Md.) using 20 ng random hexamer (Gibco) as primer. The reverse transcription reaction was carried out for 1 h at 42 C in 10 nM Tris-HCl (pH8.3), 2.5 mM MgCl₂, 50 mM KCl, DTT 0.01 M and dNTP (each 0.5 mM). A total of 30-35 PCR cycles depending on the gene amplified were performed, followed by electrophoresis on 1% agarose gel. The specific primer pairs used were as follows: for glyceraldehyde 3-phosphate dehydrogenase (GAPDH): forward primer 5' TGAAG-GTCGGAGTCAACGGATTGGT 3' (SEQ ID NO: 9), reverse primer, 5' CATGTGGGCCATGAGGTCCACCAC 3' (SEQ ID NO: 10); for Bcl-2: forward primer 5' GGTGC-CACCTGTGGTCCACCTG 3' (SEQ ID NO: 11), reverse primer 5' CTTCACTTGTGGCCCAGATAGG 3' (SEQ ID NO: 12); for Bax: forward primer 5' GAGCAGATCAT-GAAGACAGGGG 3' (SEQ ID NO: 13), reverse primer 5' CTCCAGCAAGGCCAGCGTC 3' (SEQ ID NO: 14); for Bcl-xl: forward primer 5' CAGTGAGTGAGCAGGT-GTTTTGG 3' (SEQ ID NO: 15), reverse primer 5' GTTC-CACAAAAGTATCCCAGCCG 3' (SEQ ID NO: 16).

As shown in FIG. 6, Bcl-2 was down-regulated in cells treated with tamoxifen that do not overexpress GP88 (lanes 1-4-empty vector). Bcl-2 down-regulation was blocked in cells overexpressing GP88 and treated with tamoxifen (lanes 5-8).

EXAMPLE 2

Anti-GP88 Antibody 5B4 Induces Apoptosis in Cells Overexpressing GP88 (O4 Cells) or Cells Resistant to Tamoxifen

As discussed above, cleavage of PARP releases an 85 kDa fragment indicating the cell is undergoing apoptosis. Western blot analysis using an anti-PARP antibody revealed the presence or absence of apoptosis in cells treated with combinations of tamoxifen, anti-GP88 antibody, and estradiol.

MCF-7 cells or O4 cells were seeded at a density of 7×10^5 cells in 60-mm dish in DMEM/F12 supplemented with 5% FBS. After 24 hours, medium was changed to serum-free phenol red-free DMEM/F12 supplemented with vehicle or purified GP88. After another 24 hours, cells were treated with either vehicle only or various combinations of tamoxifen, anti-GP88 antibody, estradiol, or vehicle for 24 hours. Cell lysates were collected in 6M urea in RIPA buffer (50 mM Tris HCl pH 7.4, 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM sodium orthovanadate, 1 mM NaF, protease inhibitors). 100 μ g of protein from each sample was used for immunoblotting with anti-PARP antibody. Intact and cleaved forms of PARP were detected using a mouse monoclonal anti-PARP antibody from Oncogene Research (San Diego, Calif.). α -Actin was used for normalizing the loading.

As shown in FIG. 5, GP88 inhibits induction of apoptosis by tamoxifen. Estradiol, an activator of the estrogen receptor pathway, also blocks induction of apoptosis by tamoxifen.

FIGS. 21 and 22 show that anti-GP88 antibody 5B4 induces apoptosis in cells overexpressing GP88 (O4 cells) or cells resistant to tamoxifen.

The above description and examples are only illustrative of embodiments which achieve the objects, features, and

advantages of the present invention, and it is not intended that the present invention be limited thereto. Any modifications of the present invention which come within the spirit and scope of the following claims is considered part of the present invention.

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 420 425 430
 Thr Pro Leu Gln Ile Gly Asp Ile Gly Cys Asp Gln His Thr Ser Cys
 435 440 445
 Pro Val Gly Gln Thr Cys Cys Pro Ser Leu Lys Gly Ser Trp Ala Cys
 450 455 460
 Cys Gln Leu Pro His Ala Val Cys Cys Glu Asp Arg Gln His Cys Cys
 465 470 475 480
 Pro Ala Gly Tyr Thr Cys Asn Val Lys Ala Arg Thr Cys Glu Lys Asp
 485 490 495
 Val Asp Phe Ile Gln Pro Pro Val Leu Leu Thr Leu Gly Pro Lys Val
 500 505 510
 Gly Asn Val Glu Cys Gly Glu Gly His Phe Cys His Asp Asn Gln Thr
 515 520 525
 Cys Cys Lys Asp Ser Ala Gly Val Trp Ala Cys Cys Pro Tyr Leu Lys
 530 535 540
 Gly Val Cys Cys Arg Asp Gly Arg His Cys Cys Pro Gly Gly Phe His
 545 550 555 560
 Cys Ser Ala Arg Gly Thr Lys Cys Leu Arg Lys Lys Ile Pro Arg Trp
 565 570 575
 Asp Met Phe Leu Arg Asp Pro Val Pro Arg Pro Leu Leu
 580 585

<210> SEQ ID NO 3
 <211> LENGTH: 2095
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 3

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gccagctaca gctgctgccc tccccttctg gacaaatggc ccacaacact gagcaggcat 180
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gtctcaggga cttccagttg ctgccccttc ccagaggccc tggcatgccc ggatggccat 300
cactgctgcc cacggggctt ccaactgcagt gcagacgggc gatcctgctt ccaaagatca 360
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tccacgtgct gtggttatgt cgatggctcc tgggggtgct gcccctatgcc ccaggcttcc 480
tgctgtgaag acagggtgca ctgctgtccc cacgggtgct tctgcccact gggtcacacc 540
cgctgcatca caccacggg caccacccc ctggcaaaga agctccctgc ccagaggact 600
aacagggcag tggccttgc cagctcggtc atgtgtccgg acgcacggtc ccggtgcctt 660
gatggttcta cctgctgtga gctgcccagt gggaaagtatg gctgctgccc aatgcccac 720
gccacctgct gctccgatca cctgcactgc tgcccacaag aactgtgtg tgacctgatc 780
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tgccgtctac agtcgggggc ctggggctgc tgcccttcta cccaggctgt gtgctgtgag 960
gaccacatac actgctgtcc cgcgggggtt acgtgtgaca cgcagaaggg tacctgtgaa 1020
caggggcccc accaggtgcc ctggatggag aaggccccag ctcacctcag cctgccagac 1080
ccacaagcct tgaagagaga tgtcccctgt gataatgtca gcagctgtcc ctccctcgat 1140
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gtactgaaga ctctgcagc ctcgggacc cactcggagg gtgccctctg ctcaggcctc 1860
cctagcacct ccccctaacc aaattctccc tggaccccat tctgagctcc ccatcacat 1920
gggaggtggg gcctcaatct aaggcccttc cctgtcagaa gggggttgag gcaaaagccc 1980
attacaagct gccatcccct ccccgtttca gtggacctg tggccagggtg cttttcccta 2040
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<210> SEQ ID NO 4
<211> LENGTH: 1676
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 4

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Met Glu Thr Thr Arg Pro Thr His Arg Leu Glu Val Ala Leu Ser Glu
 1             5             10             15

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Arg Thr Arg Pro Val Ala Leu Ala Leu Ala Leu Glu Thr His Arg Ala
 20             25             30

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Leu Ala Gly Leu Tyr Leu Glu Val Ala Leu Ala Leu Ala Gly Leu Tyr
35 40 45

Thr His Arg Ala Arg Gly Cys Tyr Ser Pro Arg Ala Ser Pro Gly Leu
50 55 60

Tyr Gly Leu Asn Pro His Glu Cys Tyr Ser Pro Arg Val Ala Leu Ala
65 70 75 80

Leu Ala Cys Tyr Ser Cys Tyr Ser Leu Glu Ala Ser Pro Pro Arg Gly
85 90 95

Leu Tyr Gly Leu Tyr Ala Leu Ala Ser Glu Arg Thr Tyr Arg Ser Glu
100 105 110

Arg Cys Tyr Ser Cys Tyr Ser Ala Arg Gly Pro Arg Leu Glu Leu Glu
115 120 125

Ala Ser Pro Leu Tyr Ser Thr Arg Pro Pro Arg Thr His Arg Thr His
130 135 140

Arg Leu Glu Ser Glu Arg Ala Arg Gly His Ile Ser Leu Glu Gly Leu
145 150 155 160

Tyr Gly Leu Tyr Pro Arg Cys Tyr Ser Gly Leu Asn Val Ala Leu Ala
165 170 175

Ser Pro Ala Leu Ala His Ile Ser Cys Tyr Ser Ser Glu Arg Ala Leu
180 185 190

Ala Gly Leu Tyr His Ile Ser Ser Glu Arg Cys Tyr Ser Ile Leu Glu
195 200 205

Pro His Glu Thr His Arg Val Ala Leu Ser Glu Arg Gly Leu Tyr Thr
210 215 220

His Arg Ser Glu Arg Ser Glu Arg Cys Tyr Ser Cys Tyr Ser Pro Arg
225 230 235 240

Pro His Glu Pro Arg Gly Leu Ala Leu Ala Val Ala Leu Ala Leu Ala
245 250 255

Cys Tyr Ser Gly Leu Tyr Ala Ser Pro Gly Leu Tyr His Ile Ser His
260 265 270

Ile Ser Cys Tyr Ser Cys Tyr Ser Pro Arg Ala Arg Gly Gly Leu Tyr
275 280 285

Pro His Glu His Ile Ser Cys Tyr Ser Ser Glu Arg Ala Leu Ala Ala
290 295 300

Ser Pro Gly Leu Tyr Ala Arg Gly Ser Glu Arg Cys Tyr Ser Pro His
305 310 315 320

Glu Gly Leu Asn Ala Arg Gly Ser Glu Arg Gly Leu Tyr Ala Ser Asn
325 330 335

Ala Ser Asn Ser Glu Arg Val Ala Leu Gly Leu Tyr Ala Leu Ala Ile
340 345 350

Leu Glu Gly Leu Asn Cys Tyr Ser Pro Arg Ala Ser Pro Ser Glu Arg
355 360 365

Gly Leu Asn Pro His Glu Gly Leu Cys Tyr Ser Pro Arg Ala Ser Pro
370 375 380

Pro His Glu Ser Glu Arg Thr His Arg Cys Tyr Ser Cys Tyr Ser Val
385 390 395 400

Ala Leu Met Glu Thr Val Ala Leu Ala Ser Pro Gly Leu Tyr Ser Glu
405 410 415

Arg Thr Arg Pro Gly Leu Tyr Cys Tyr Ser Cys Tyr Ser Pro Arg Met
420 425 430

Glu Thr Pro Arg Gly Leu Asn Ala Leu Ala Ser Glu Arg Cys Tyr Ser
435 440 445

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Cys Tyr Ser Gly Leu Ala Ser Pro Ala Arg Gly Val Ala Leu His Ile
 450 455 460
 Ser Cys Tyr Ser Cys Tyr Ser Pro Arg His Ile Ser Gly Leu Tyr Ala
 465 470 475 480
 Leu Ala Pro His Glu Cys Tyr Ser Ala Ser Pro Leu Glu Val Ala Leu
 485 490 495
 His Ile Ser Thr His Arg Ala Arg Gly Cys Tyr Ser Ile Leu Glu Thr
 500 505 510
 His Arg Pro Arg Thr His Arg Gly Leu Tyr Thr His Arg His Ile Ser
 515 520 525
 Pro Arg Leu Glu Ala Leu Ala Leu Tyr Ser Leu Tyr Ser Leu Glu Pro
 530 535 540
 Arg Ala Leu Ala Gly Leu Asn Ala Arg Gly Thr His Arg Ala Ser Asn
 545 550 555 560
 Ala Arg Gly Ala Leu Ala Val Ala Leu Ala Leu Ala Leu Glu Ser Glu
 565 570 575
 Arg Ser Glu Arg Ser Glu Arg Val Ala Leu Met Glu Thr Cys Tyr Ser
 580 585 590
 Pro Arg Ala Ser Pro Ala Leu Ala Ala Arg Gly Ser Glu Arg Ala Arg
 595 600 605
 Gly Cys Tyr Ser Pro Arg Ala Ser Pro Gly Leu Tyr Ser Glu Arg Thr
 610 615 620
 His Arg Cys Tyr Ser Cys Tyr Ser Gly Leu Leu Glu Pro Arg Ser Glu
 625 630 635 640
 Arg Gly Leu Tyr Leu Tyr Ser Thr Tyr Arg Gly Leu Tyr Cys Tyr Ser
 645 650 655
 Cys Tyr Ser Pro Arg Met Glu Thr Pro Arg Ala Ser Asn Ala Leu Ala
 660 665 670
 Thr His Arg Cys Tyr Ser Cys Tyr Ser Ser Glu Arg Ala Ser Pro His
 675 680 685
 Ile Ser Leu Glu His Ile Ser Cys Tyr Ser Cys Tyr Ser Pro Arg Gly
 690 695 700
 Leu Asn Ala Ser Pro Thr His Arg Val Ala Leu Cys Tyr Ser Ala Ser
 705 710 715 720
 Pro Leu Glu Ile Leu Glu Gly Leu Asn Ser Glu Arg Leu Tyr Ser Cys
 725 730 735
 Tyr Ser Leu Glu Ser Glu Arg Leu Tyr Ser Gly Leu Ala Ser Asn Ala
 740 745 750
 Leu Ala Thr His Arg Thr His Arg Ala Ser Pro Leu Glu Leu Glu Thr
 755 760 765
 His Arg Leu Tyr Ser Leu Glu Pro Arg Ala Leu Ala His Ile Ser Thr
 770 775 780
 His Arg Val Ala Leu Gly Leu Tyr Ala Ser Pro Val Ala Leu Leu Tyr
 785 790 795 800
 Ser Cys Tyr Ser Ala Ser Pro Met Glu Thr Gly Leu Val Ala Leu Ser
 805 810 815
 Glu Arg Cys Tyr Ser Pro Arg Ala Ser Pro Gly Leu Tyr Thr Tyr Arg
 820 825 830
 Thr His Arg Cys Tyr Ser Cys Tyr Ser Ala Arg Gly Leu Glu Gly Leu
 835 840 845
 Asn Ser Glu Arg Gly Leu Tyr Ala Leu Ala Thr Arg Pro Gly Leu Tyr
 850 855 860
 Cys Tyr Ser Cys Tyr Ser Pro Arg Pro His Glu Thr His Arg Gly Leu

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865	870	875	880
Asn Ala Leu Ala Val Ala Leu Cys Tyr Ser Cys Tyr Ser Gly Leu Ala	885	890	895
Ser Pro His Ile Ser Ile Leu Glu His Ile Ser Cys Tyr Ser Cys Tyr	900	905	910
Ser Pro Arg Ala Leu Ala Gly Leu Tyr Pro His Glu Thr His Arg Cys	915	920	925
Tyr Ser Ala Ser Pro Thr His Arg Gly Leu Asn Leu Tyr Ser Gly Leu	930	935	940
Tyr Thr His Arg Cys Tyr Ser Gly Leu Gly Leu Asn Gly Leu Tyr Pro	945	950	955
Arg His Ile Ser Gly Leu Asn Val Ala Leu Pro Arg Thr Arg Pro Met	965	970	975
Glu Thr Gly Leu Leu Tyr Ser Ala Leu Ala Pro Arg Ala Leu Ala His	980	985	990
Ile Ser Leu Glu Ser Glu Arg Leu Glu Pro Arg Ala Ser Pro Pro Arg	995	1000	1005
Gly Leu Asn Ala Leu Ala Leu Glu Leu Tyr Ser Ala Arg Gly Ala Ser	1010	1015	1020
Pro Val Ala Leu Pro Arg Cys Tyr Ser Ala Ser Pro Ala Ser Asn Val	1025	1030	1035
Ala Leu Ser Glu Arg Ser Glu Arg Cys Tyr Ser Pro Arg Ser Glu Arg	1045	1050	1055
Ser Glu Arg Ala Ser Pro Thr His Arg Cys Tyr Ser Cys Tyr Ser Gly	1060	1065	1070
Leu Asn Leu Glu Thr His Arg Ser Glu Arg Gly Leu Tyr Gly Leu Thr	1075	1080	1085
Arg Pro Gly Leu Tyr Cys Tyr Ser Cys Tyr Ser Pro Arg Ile Leu Glu	1090	1095	1100
Pro Arg Gly Leu Ala Leu Ala Val Ala Leu Cys Tyr Ser Cys Tyr Ser	1105	1110	1115
Ser Glu Arg Ala Ser Pro His Ile Ser Gly Leu Asn His Ile Ser Cys	1125	1130	1135
Tyr Ser Cys Tyr Ser Pro Arg Gly Leu Asn Ala Arg Gly Thr Tyr Arg	1140	1145	1150
Thr His Arg Cys Tyr Ser Val Ala Leu Ala Leu Ala Gly Leu Gly Leu	1155	1160	1165
Tyr Gly Leu Asn Cys Tyr Ser Gly Leu Asn Ala Arg Gly Gly Leu Tyr	1170	1175	1180
Ser Glu Arg Gly Leu Ile Leu Glu Val Ala Leu Ala Leu Ala Gly Leu	1185	1190	1195
Tyr Leu Glu Gly Leu Leu Tyr Ser Met Glu Thr Pro Arg Ala Leu Ala	1205	1210	1215
Ala Arg Gly Ala Arg Gly Gly Leu Tyr Ser Glu Arg Leu Glu Ser Glu	1220	1225	1230
Arg His Ile Ser Pro Arg Ala Arg Gly Ala Ser Pro Ile Leu Glu Gly	1235	1240	1245
Leu Tyr Cys Tyr Ser Ala Ser Pro Gly Leu Asn His Ile Ser Thr His	1250	1255	1260
Arg Ser Glu Arg Cys Tyr Ser Pro Arg Val Ala Leu Gly Leu Tyr Gly	1265	1270	1275
Leu Tyr Thr His Arg Cys Tyr Ser Cys Tyr Ser Pro Arg Ser Glu Arg	1285	1290	1295

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Gly Leu Asn Gly Leu Tyr Gly Leu Tyr Ser Glu Arg Thr Arg Pro Ala
 1300 1305 1310

Leu Ala Cys Tyr Ser Cys Tyr Ser Gly Leu Asn Leu Glu Pro Arg His
 1315 1320 1325

Ile Ser Ala Leu Ala Val Ala Leu Cys Tyr Ser Cys Tyr Ser Gly Leu
 1330 1335 1340

Ala Ser Pro Ala Arg Gly Gly Leu Asn His Ile Ser Cys Tyr Ser Cys
 1345 1350 1355 1360

Tyr Ser Pro Arg Ala Leu Ala Gly Leu Tyr Thr Tyr Arg Thr His Arg
 1365 1370 1375

Cys Tyr Ser Ala Ser Asn Val Ala Leu Leu Tyr Ser Ala Leu Ala Ala
 1380 1385 1390

Arg Gly Ser Glu Arg Cys Tyr Ser Gly Leu Leu Tyr Ser Gly Leu Val
 1395 1400 1405

Ala Leu Val Ala Leu Ser Glu Arg Ala Leu Ala Gly Leu Asn Pro Arg
 1410 1415 1420

Ala Leu Ala Thr His Arg Pro His Glu Leu Glu Ala Leu Ala Ala Arg
 1425 1430 1435 1440

Gly Ser Glu Arg Pro Arg His Ile Ser Val Ala Leu Gly Leu Tyr Val
 1445 1450 1455

Ala Leu Leu Tyr Ser Ala Ser Pro Val Ala Leu Gly Leu Cys Tyr Ser
 1460 1465 1470

Gly Leu Tyr Gly Leu Gly Leu Tyr His Ile Ser Pro His Glu Cys Tyr
 1475 1480 1485

Ser His Ile Ser Ala Ser Pro Ala Ser Asn Gly Leu Asn Thr His Arg
 1490 1495 1500

Cys Tyr Ser Cys Tyr Ser Ala Arg Gly Ala Ser Pro Ala Ser Asn Ala
 1505 1510 1515 1520

Arg Gly Gly Leu Asn Gly Leu Tyr Thr Arg Pro Ala Leu Ala Cys Tyr
 1525 1530 1535

Ser Cys Tyr Ser Pro Arg Thr Tyr Arg Ala Leu Ala Gly Leu Asn Gly
 1540 1545 1550

Leu Tyr Val Ala Leu Cys Tyr Ser Cys Tyr Ser Ala Leu Ala Ala Ser
 1555 1560 1565

Pro Ala Arg Gly Ala Arg Gly His Ile Ser Cys Tyr Ser Cys Tyr Ser
 1570 1575 1580

Pro Arg Ala Leu Ala Gly Leu Tyr Pro His Glu Ala Arg Gly Cys Tyr
 1585 1590 1595 1600

Ser Ala Leu Ala Ala Arg Gly Ala Arg Gly Gly Leu Tyr Thr His Arg
 1605 1610 1615

Leu Tyr Ser Cys Tyr Ser Leu Glu Ala Arg Gly Ala Arg Gly Gly Leu
 1620 1625 1630

Ala Leu Ala Pro Arg Ala Arg Gly Thr Arg Pro Ala Ser Pro Ala Leu
 1635 1640 1645

Ala Pro Arg Leu Glu Ala Arg Gly Ala Ser Pro Pro Arg Ala Leu Ala
 1650 1655 1660

Leu Glu Ala Arg Gly Gly Leu Asn Leu Glu Leu Glu
 1665 1670 1675

<210> SEQ ID NO 5
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 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide sequence

<400> SEQUENCE: 5

aggttgatgc cactgctct g 21

<210> SEQ ID NO 6

<211> LENGTH: 21

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide sequence

<400> SEQUENCE: 6

gagcaguggg caucaaccug g 21

<210> SEQ ID NO 7

<211> LENGTH: 21

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide sequence

<400> SEQUENCE: 7

agatcaggta acaactccgt g 21

<210> SEQ ID NO 8

<211> LENGTH: 21

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide sequence

<400> SEQUENCE: 8

ggacacttct gccatgataa c 21

<210> SEQ ID NO 9

<211> LENGTH: 26

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic PCR primer

<400> SEQUENCE: 9

tgaaggtcgg agtcaacgga tttggt 26

<210> SEQ ID NO 10

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic PCR primer

<400> SEQUENCE: 10

catgtgggcc atgaggtcca ccac 24

<210> SEQ ID NO 11

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic

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PCR primer

<400> SEQUENCE: 11

ggtgccacct gtggtccacc tg 22

<210> SEQ ID NO 12
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 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
 PCR primer

<400> SEQUENCE: 12

cttcacttgt ggcccagata gg 22

<210> SEQ ID NO 13
 <211> LENGTH: 22
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
 PCR primer

<400> SEQUENCE: 13

gagcagatca tgaagacagg gg 22

<210> SEQ ID NO 14
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
 PCR primer

<400> SEQUENCE: 14

ctccagcaag gcccagcgtc 20

<210> SEQ ID NO 15
 <211> LENGTH: 23
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
 PCR primer

<400> SEQUENCE: 15

cagtgagtga gcaggtgttt tgg 23

<210> SEQ ID NO 16
 <211> LENGTH: 23
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
 PCR primer

<400> SEQUENCE: 16

gttccacaaa agtatcccag ccg 23

<210> SEQ ID NO 17
 <211> LENGTH: 55
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Consensus
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<220> FEATURE:
<221> NAME/KEY: MOD_RES
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<223> OTHER INFORMATION: Variable amino acid
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<223> OTHER INFORMATION: Variable amino acid
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<222> LOCATION: (16)..(20)
<223> OTHER INFORMATION: Variable amino acid
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<223> OTHER INFORMATION: Variable amino acid
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<222> LOCATION: (28)..(33)
<223> OTHER INFORMATION: Variable amino acid
<220> FEATURE:
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<222> LOCATION: (36)
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<223> OTHER INFORMATION: Variable amino acid
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<223> OTHER INFORMATION: Variable amino acid
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<222> LOCATION: (49)..(54)
<223> OTHER INFORMATION: Variable amino acid

<400> SEQUENCE: 17

Cys Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Cys Cys Xaa
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Xaa Xaa Xaa Xaa Gly Xaa Xaa Xaa Xaa Cys Cys Xaa Xaa Xaa Xaa Xaa
 20                               25                               30

Xaa Cys Cys Xaa Asp Xaa Xaa His Cys Cys Pro Xaa Xaa Xaa Xaa Cys
 35                               40                               45

Xaa Xaa Xaa Xaa Xaa Xaa Cys
 50                               55

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What is claimed as new and desired to be protected by Letters Patent of the United States is:

1. A method for restoring sensitivity to antitumorigenic effects of antiestrogen therapy, comprising contacting a breast tumor cell that is nonsensitive to the antitumorigenic effects of antiestrogen therapy with a PCDGF antagonist in an amount effective to restore sensitivity to antitumorigenic effects of antiestrogen therapy, wherein the PCDGF antagonist is an antibody or antibody fragment thereof produced from a hybridoma cell line selected from the group consisting of 6B3 hybridoma cell line (ATCC Accession Number PTA-5262), 6B2 hybridoma cell line (ATCC Accession Number PTA-5261), 6C12 hybridoma cell line (ATCC Accession Number PTA-5597), 5B4 hybridoma cell line (ATCC Accession Number PTA-5260), 5G6 hybridoma cell line (ATCC Accession Number PTA-5595), 4D1 hybridoma cell line (ATCC Accession Number PTA-5593), 3F8 hybridoma cell line (ATCC Accession Number PTA-5591), 3F5 hybridoma cell line (ATCC Accession Number PTA-5259), 3F4 hybridoma cell line (ATCC Accession Number PTA-

45

5590), 3G2 (ATCC Accession Number PTA-5592), and 2A5 hybridoma cell line (ATCC Accession Number PTA-5589).

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2. The method of claim 1, wherein the antiestrogen therapy is selected from the group consisting of tamoxifen, raloxifene, aromatase inhibitors, and estrogen receptor down-regulators.

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3. A method of inducing apoptosis in a tumor cell, comprising contacting a PCDGF antagonist to a tumor cell in an amount effective to induce apoptosis of the tumor cell, wherein the PCDGF antagonist is an antibody or antibody fragment thereof produced from a hybridoma cell line selected from the group consisting of 6B3 hybridoma cell line (ATCC Accession Number PTA-5262), 6B2 hybridoma cell line (ATCC Accession Number PTA-5261), 6C12 hybridoma cell line (ATCC Accession Number PTA-5597), 5B4 hybridoma cell line (ATCC Accession Number PTA-5260), 5G6 hybridoma cell line (ATCC Accession Number PTA-5595): 4D1 hybridoma cell line (ATCC Accession Number PTA-5593), 3F8 hybridoma cell line (ATCC Accession Number PTA-5591), 3F5 hybridoma cell line (ATCC Accession Number PTA-5259), 3F4 hybridoma cell line (ATCC

49

Accession Number PTA-5590): 3G2 (ATCC Accession Number PTA-5592), and 2A5 hybridoma cell line (ATCC Accession Number PTA-5589).

4. The method of claim 3, wherein the tumor cell is selected from the group consisting of prostate, head and neck, neural, nasopharynx, thyroid, bladder, cervix, colorectal, blood, liver, kidney, breast, bone, pancreas, bone marrow, testes, ovaries, brain, neural, colon, and lung tumors.

5. A method of inducing apoptosis in a tumor cell, comprising contacting a PCDGF antagonist and an anti-estrogen with the tumor cell to induce apoptosis in the tumor cell—wherein the PCDGF antagonist is an antibody or antibody fragment thereof produced from a hybridoma cell line selected from the group consisting of 6B3 hybridoma cell line (ATCC Accession Number PTA-5262), 6B2 hybridoma cell line (ATCC Accession Number PTA-5261), 6C 12 hybridoma cell line (ATCC Accession Number PTA-5597), 5B4 hybridoma cell line (ATCC Accession Number PTA-5260), 5G6 hybridoma cell line (ATCC Accession Number

50

PTA-5595), 4D1 hybridoma cell line (ATCC Accession Number PTA-5593), 3F8 hybridoma cell line (ATCC Accession Number PTA-5591), 3F5 hybridoma cell line (ATCC Accession Number PTA-5259), 3F4 hybridoma cell line (ATCC Accession Number PTA-5590), 3G2 (ATCC Accession Number PTA-5592), and 2A5 hybridoma cell line (ATCC Accession Number PTA-5589).

6. The method of claim 5, wherein the anti-estrogen is selected from group consisting of tamoxifen, raloxifene, aromatase inhibitors, and estrogen receptor down-regulators.

7. The method of claim 5 wherein the tumor cell is from a tumor selected from the group consisting of prostate, head and neck, nasopharynx, cervix, colorectal, bladder, thyroid, pancreas, blood, liver, kidney, breast, bone, bone marrow, testes, ovaries, brain, neural, colon, and lung tumors.

* * * * *